PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
WOB 98 AS IDM TID		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/08980	22/11/1999	02/12/1998
Applicant		
I.D.M. IMMUNO-DESIGNED MOLECULES et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

onal application No.
CT/EP 99/08980

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The conjugate according to claims 1-8 is not clear in view of the broad and vague definition of both the "monomeric component" and the "protonable residues". The search had to be restricted for economic reasons and was limited to the worked examples and to the general idea underlying the application.
See Articles 5 and 6 PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The invention relates to a positively charged oligomeric conjugate, containing an oligomer with a polymerization degree (PD) from 5 to 50, preferably 10 to 40 and more preferably 20, formed from monomeric components having free NH_3^+ in a number equal to or higher than 50% of the polymerization degree.
In particular, the invention provides new oligomeric conjugates of histidylated oligolysine liable to allow the transfer of oligonucleotides, peptides and oligosides into cells.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]EP 99/08980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/11 A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 22610 A (IDM IMMUNO DESIGNED MOLECULES ;MIDOUX PATRICK (FR); MONSIGNY MICHE) 28 May 1998 (1998-05-28) cited in the application * see in particular the claims; and Figures 1,5 *	1-7,9-19
A	WO 98 19710 A (SCHACHT ETIENNE HONORE ;ULBRICH KAREL (CZ); SEYMOUR LEONARD CHARLE) 14 May 1998 (1998-05-14) *see p.39,l.30 - p.40; claims 1,6-8*	1-19
A	US 5 627 270 A (HATZENBUHLER NICOLE T ET AL) 6 May 1997 (1997-05-06) * see claims 1,3; col.71,l.36-38 *	1-19

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2000	17.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Isert, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED T BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 727 223 A (HISAMITSU PHARMACEUTICAL CO) 21 August 1996 (1996-08-21) * see the claims; Figures 8-10; page 7 *	1-19
A	GOTO T. ET AL: "A novel approach for gene medicine;synthetic poly-L- lysine /serine copolymer enhances bioactivity of antisense oligonucleotides." NUCLEOSIDES AND NUCLEOTIDES, (1997) 16/7-9 (1609-1615). REFS: 10 ISSN: 0732-8311 CODEN: NUNUD5, XP002108844 United States *see in particular abstract and introduction *	1-19
A	MIDOUX P ET AL: "Membrane permeabilization and efficient gene transfer by a peptide containing several histidines." BIOCONJUGATE CHEMISTRY, (1998 MAR-APR) 9 (2) 260-7. JOURNAL CODE: A1T. ISSN: 1043-1802., XP002108845 United States *see in particular the abstract*	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
EP 99/08980

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9822610	A	28-05-1998	FR	2755976 A	22-05-1998
			AU	5123998 A	10-06-1998
			EP	0946744 A	06-10-1999
WO 9819710	A	14-05-1998	AU	4873997 A	29-05-1998
			EP	0941123 A	15-09-1999
US 5627270	A	06-05-1997	US	5693769 A	02-12-1997
			US	5571795 A	05-11-1996
			US	5338837 A	16-08-1994
			AU	687557 B	26-02-1998
			AU	2358295 A	16-11-1995
			CA	2188320 A	02-11-1995
			EP	0756601 A	05-02-1997
			JP	9512270 T	09-12-1997
			NZ	284902 A	22-09-1997
			WO	9529186 A	02-11-1995
			US	5795870 A	18-08-1998
			US	5780444 A	14-07-1997
			AT	166577 T	15-06-1998
			AU	3278593 A	19-07-1993
			BR	9206927 A	21-11-1995
			CA	2117332 A	24-06-1993
			DE	69225719 D	02-07-1998
			DE	69225719 T	24-12-1998
			EP	0618800 A	12-10-1994
			ES	2118932 T	01-10-1998
			HU	70743 A	30-10-1995
			IL	104089 A	09-05-1999
			JP	7503708 T	20-04-1995
			NO	942165 A	01-08-1994
			NZ	246448 A	28-07-1998
			PL	171131 B	28-03-1997
			WO	9311772 A	24-06-1993
			US	5455335 A	03-10-1996
EP 0727223	A	21-08-1996	AU	681192 B	21-08-1997
			AU	7706994 A	18-04-1995
			US	5912300 A	15-06-1999
			CA	2172974 A	06-04-1995
			WO	9509009 A	06-04-1995

ATENT COOPERATION TRE.

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 June 2000 (13.06.00)	
International application No. PCT/EP99/08980	**Applicant's or agent's file reference** WOB 98 AS IDM TID
International filing date (day/month/year) 22 November 1999 (22.11.99)	**Priority date** (day/month/year) 02 December 1998 (02.12.98)
Applicant MIDOUX, Patrick et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 May 2000 (02.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	*Authorized officer* S. Mafla
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

REC'D 0 1 FEB 2001
WIPO PCT

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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WOB 98 AS IDM TID		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08980	22/11/1999	02/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/11		
Applicant I.D.M. IMMUNO-DESIGNED MOLECULES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/05/2000	29.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Isert, B Telephone No. +49 89 2399 8691

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08980

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-39 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-19 (in part).

because:

   ☒ the said international application, or the said claims Nos. 11-16 (for industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-19 (in part).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-19 (see Separate Sheet, item 2)

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19 (see Separate Sheet, item 2)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	see remark on Separate Sheet

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION III

1). Claims 11-16 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

2). Claims 1-19 are examined on matter which has been searched, that is the use of histidylated oligolysine in the gene transfer of an oligonucleotide, cf. page 2 and page 38, line 23 - page 39. See the remarks made on form PCT/ISA/210.

SECTION V:

3). The following documents (D) cited in the International search report are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 = WO-A-9822610 (also cited in the application)
D2 = WO-A-9819710
D3 = US-A-5627270
D4 = EP-A-0727223
D5 = Nucleosides and Nucleotides, 1997, 16 (7-9): 1609-1615
D6 = Bioconjugate Chemistry, 1998, 9 (2): 260-267

Unless indicated otherwise reference is made to the relevant passages emphasized in the search report.

4). Novelty:

The histidylated oligolysines according to the present examples are novel over the substituted polylysines described in D1, D2, D4, and D5.
Oligolysines having a DP of 5 to 50 substituted with more than 50% histidyl residues could be considered a selection over the disclosure of D1 (DP: 15 to 900,

at least 10 % substitution) in that there is no overlap with the preferred scope of D1 (cf. page 15, DP: 100 to 300, 10 % to 45 % substitution).

5). Inventive step:

The present application relates to the transfer of oligonucleotides, peptides and oligosides into cells. As it is shown in Table 1, the efficient transfer of oligonucleotides can be achieved in the presence of histidylated oligolysines (DP of 36 or 19, substituted by more than 50% histidyl residues).
D4 and D5 also concern the transfer of oligonucleotides, wherein complexes with polylysine-serine copolymers have been used. In particular, Figures 8-10 of D4 show enhanced transfer brought about by the copolymers in comparison to polylysine (molecular weights 3000 - 37200). The present oligolysines differ therefrom in particular by the substitution with histidyl residues. D6 (and D1) point to the importance of histidine residues as helpers for the delivery of molecules into the cell. However, the use of oligolysines having a particular amount of histidine substituents would not be considered obvious.

6). Industrial applicability

For the assessment of the present claims 11-16 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The compositions of claims 1-10 having a pharmaceutical use are considered industrially applicable under Article 33 (4) PCT.

SECTION VII

7). Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2,D4 and D6 is not mentioned in the description, nor are these documents identified therein.

SECTION VIII

8). In view of the limitation of the search carried out, no further remarks are made upon the conjugates of claims 1-8.

9). The term "biological molecule" used in claims 9-11,19 allows for ambiguous interpretation and should be replaced by the term "peptide, oligoside or an oligonucleotide".

10). Claims 17 and 18 are not clear in that the conjugates are not the active agents for treating disease conditions, but rather the conjugates in association with the oligonucleotides etc, are used therefor.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
WOB 98 AS IDM TID		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/08980	22/11/1999	02/12/1998

International Patent Classification (IPC) or national classification and IPC
C12N15/11

Applicant
I.D.M. IMMUNO-DESIGNED MOLECULES et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/05/2000	29.01.2001

Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Isert, B Telephone No. +49 89 2399 8691

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08980

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-39 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-19 (in part).

because:

   ☒ the said international application, or the said claims Nos. 11-16 (for industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-19 (in part).

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-19 (see Separate Sheet, item 2)

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-19 (see Separate Sheet, item 2)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	see remark on Separate Sheet

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION III

1). Claims 11-16 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

2). Claims 1-19 are examined on matter which has been searched, that is the use of histidylated oligolysine in the gene transfer of an oligonucleotide, cf. page 2 and page 38, line 23 - page 39. See the remarks made on form PCT/ISA/210.

SECTION V:

3). The following documents (D) cited in the International search report are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 = WO-A-9822610 (also cited in the application)
D2 = WO-A-9819710
D3 = US-A-5627270
D4 = EP-A-0727223
D5 = Nucleosides and Nucleotides, 1997, 16 (7-9): 1609-1615
D6 = Bioconjugate Chemistry, 1998, 9 (2): 260-267

Unless indicated otherwise reference is made to the relevant passages emphasized in the search report.

4). Novelty:

The histidylated oligolysines according to the present examples are novel over the substituted polylysines described in D1, D2, D4, and D5.
Oligolysines having a DP of 5 to 50 substituted with more than 50% histidyl residues could be considered a selection over the disclosure of D1 (DP: 15 to 900,

at least 10 % substitution) in that there is no overlap with the preferred scope of D1 (cf. page 15, DP: 100 to 300, 10 % to 45 % substitution).

5). Inventive step:

The present application relates to the transfer of oligonucleotides, peptides and oligosides into cells. As it is shown in Table 1, the efficient transfer of oligonucleotides can be achieved in the presence of histidylated oligolysines (DP of 36 or 19, substituted by more than 50% histidyl residues).
D4 and D5 also concern the transfer of oligonucleotides, wherein complexes with polylysine-serine copolymers have been used. In particular, Figures 8-10 of D4 show enhanced transfer brought about by the copolymers in comparison to polylysine (molecular weights 3000 - 37200). The present oligolysines differ therefrom in particular by the substitution with histidyl residues. D6 (and D1) point to the importance of histidine residues as helpers for the delivery of molecules into the cell. However, the use of oligolysines having a particular amount of histidine substituents would not be considered obvious.

6). Industrial applicability

For the assessment of the present claims 11-16 on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

The compositions of claims 1-10 having a pharmaceutical use are considered industrially applicable under Article 33 (4) PCT.

SECTION VII

7). Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2,D4 and D6 is not mentioned in the description, nor are these documents identified therein.

SECTION VIII

8). In view of the limitation of the search carried out, no further remarks are made upon the conjugates of claims 1-8.

9). The term "biological molecule" used in claims 9-11,19 allows for ambiguous interpretation and should be replaced by the term "peptide, oligoside or an oligonucleotide".

10). Claims 17 and 18 are not clear in that the conjugates are not the active agents for treating disease conditions, but rather the conjugates in association with the oligonucleotides etc, are used therefor.

[]0898 AS IDT

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 40 3015

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X,D	WO 98 22610 A (IDM IMMUNO DESIGNED MOLECULES ;MIDOUX PATRICK (FR); MONSIGNY MICHE) 28 May 1998 (1998-05-28) * see in particular the claims; and Figures 1,5 *	1-7,9-19	C12N15/11 A61K47/48 A61K48/00
A	WO 98 19710 A (SCHACHT ETIENNE HONORE ;ULBRICH KAREL (CZ); SEYMOUR LEONARD CHARLE) 14 May 1998 (1998-05-14) *see p.39,l.30 - p.40; claims 1,6-8*	1-19	
A	US 5 627 270 A (HATZENBUHLER NICOLE T ET AL) 6 May 1997 (1997-05-06) * see claims 1,3; col.71,l.36-38 *	1-19	
A	EP 0 727 223 A (HISAMITSU PHARMACEUTICAL CO) 21 August 1996 (1996-08-21) * see the claims; Figures 8-10; page 7 *	1-19	

-/--

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A61K

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
MUNICH	20 July 1999	ISERT B.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C07)

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 40 3015

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	GOTO T. ET AL: "A novel approach for gene medicine;synthetic poly-L- lysine /serine copolymer enhances bioactivity of antisense oligonucleotides." NUCLEOSIDES AND NUCLEOTIDES, (1997) 16/7-9 (1609-1615). REFS: 10 ISSN: 0732-8311 CODEN: NUNUD5, XP002108844 United States *see in particular abstract and introduction * ---	1-19	
A	MIDOUX P ET AL: "Membrane permeabilization and efficient gene transfer by a peptide containing several histidines." BIOCONJUGATE CHEMISTRY, (1998 MAR-APR) 9 (2) 260-7. JOURNAL CODE: A1T. ISSN: 1043-1802., XP002108845 United States *see in particular the abstract* -----	1-19	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

INTERNATIONAL SEARCH REPORT

International Application No
EP 99/08980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/11 A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 22610 A (IDM IMMUNO DESIGNED MOLECULES ;MIDOUX PATRICK (FR); MONSIGNY MICHE) 28 May 1998 (1998-05-28) cited in the application * see in particular the claims; and Figures 1,5 * ---	1-7,9-19
A	WO 98 19710 A (SCHACHT ETIENNE HONORE ;ULBRICH KAREL (CZ); SEYMOUR LEONARD CHARLE) 14 May 1998 (1998-05-14) *see p.39,l.30 - p.40; claims 1,6-8* ---	1-19
A	US 5 627 270 A (HATZENBUHLER NICOLE T ET AL) 6 May 1997 (1997-05-06) * see claims 1,3; col.71,l.36-38 * --- -/--	1-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2000	17.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Isert, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 727 223 A (HISAMITSU PHARMACEUTICAL CO) 21 August 1996 (1996-08-21) * see the claims; Figures 8-10; page 7 * ---	1-19
A	GOTO T. ET AL: "A novel approach for gene medicine;synthetic poly-L- lysine /serine copolymer enhances bioactivity of antisense oligonucleotides." NUCLEOSIDES AND NUCLEOTIDES, (1997) 16/7-9 (1609-1615). REFS: 10 ISSN: 0732-8311 CODEN: NUNUD5, XP002108844 United States *see in particular abstract and introduction * ---	1-19
A	MIDOUX P ET AL: "Membrane permeabilization and efficient gene transfer by a peptide containing several histidines." BIOCONJUGATE CHEMISTRY, (1998 MAR-APR) 9 (2) 260-7. JOURNAL CODE: A1T. ISSN: 1043-1802., XP002108845 United States *see in particular the abstract* -----	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P[redacted]P 99/08980

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9819710 A	14-05-1998	AU 4873997 A	29-05-1998
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		WO 9509009 A	06-04-1995

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/11, A61K 47/48, 48/00

A1

(11) International Publication Number: WO 00/32764

(43) International Publication Date: 8 June 2000 (08.06.00)

(21) International Application Number: PCT/EP99/08980

(22) International Filing Date: 22 November 1999 (22.11.99)

(30) Priority Data:
98 403 015.5 2 December 1998 (02.12.98) EP

(71) Applicant *(for all designated States except US)*: I.D.M. IMMUNO-DESIGNED MOLECULES [FR/FR]; 172, rue de Charonne, F-75011 Paris (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MIDOUX, Patrick [FR/FR]; 21, rue du Poinçon, F-45100 Orléans (FR). PICHON, Chantal [FR/FR]; 1358, route d'Orléans, F-45640 Sandillon (FR). BELLO-ROUFAÏ, Mahajoub [FR/FR]; 5, rue de Tours, Résidence Les Hêtres, F-45072 Orléans Cedex 02 (FR). MONSIGNY, Michel [FR/FR]; 341, rue des Bouvreuils, F-45590 Saint-Cyr-en-Val (FR).

(74) Agents: GROSSET-FOURNIER, Chantal et al.; Grosset-Fournier & Demachy, 20, rue de Maubeuge, F-75009 Paris (FR).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NEW OLIGOMERIC CONJUGATES LIABLE TO TRANSFER BIOLOGICAL MOLECULES INTO CELLS

(57) Abstract

The invention relates to a positively charged oligomeric conjugate, containing an oligomer with a polymerization degree (PD) from 5 to 50, preferably 10 to 40 and more preferably 20, formed from monomeric components having free NH_3^+ in a number equal to or higher than 50 % of the polymerization degree. In particular, the invention provides new oligomeric conjugates of histidylated oligolysine liable to allow the transfer of oligonucleotides, peptides and oligosides into cells.

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DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

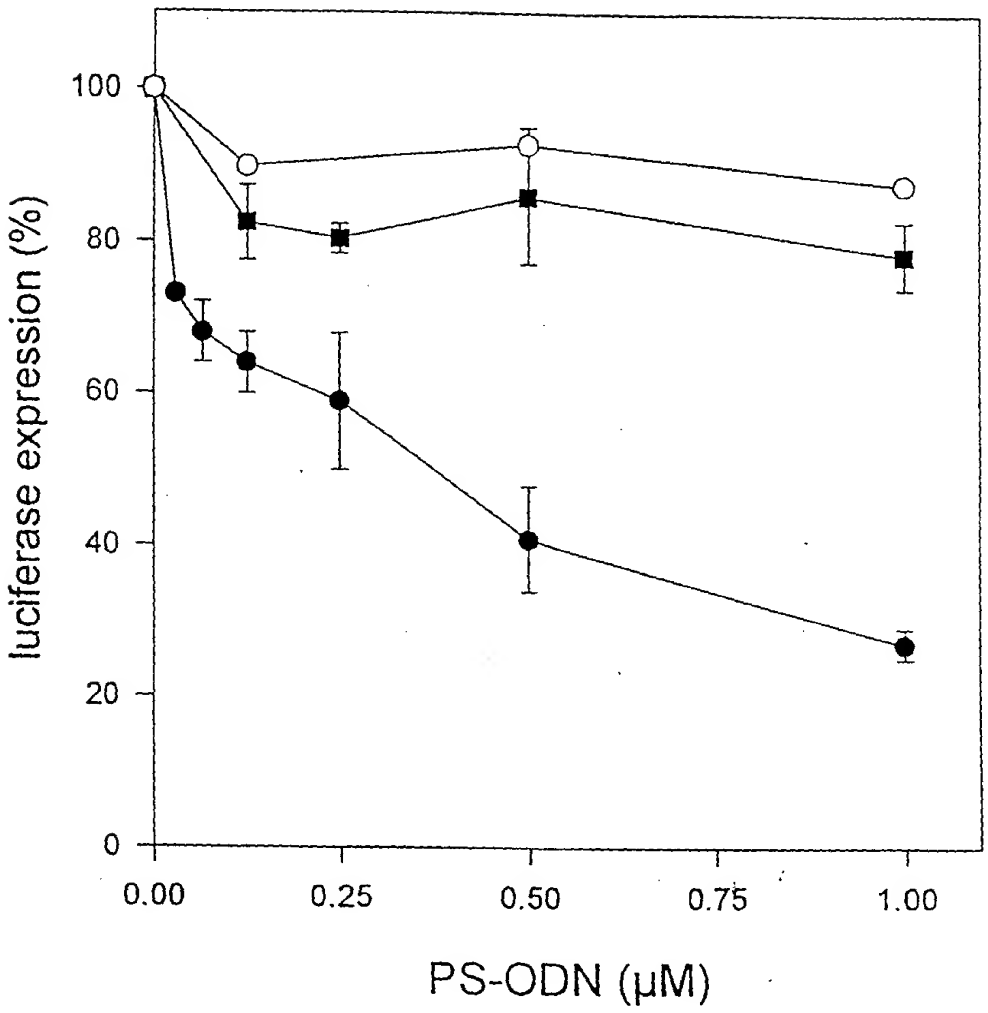
100
80
60
40
20
0
luciferase expression (%)
0.00
0.25
0.50
0.75
1.00
PS-ODN (µM)

Figure 1

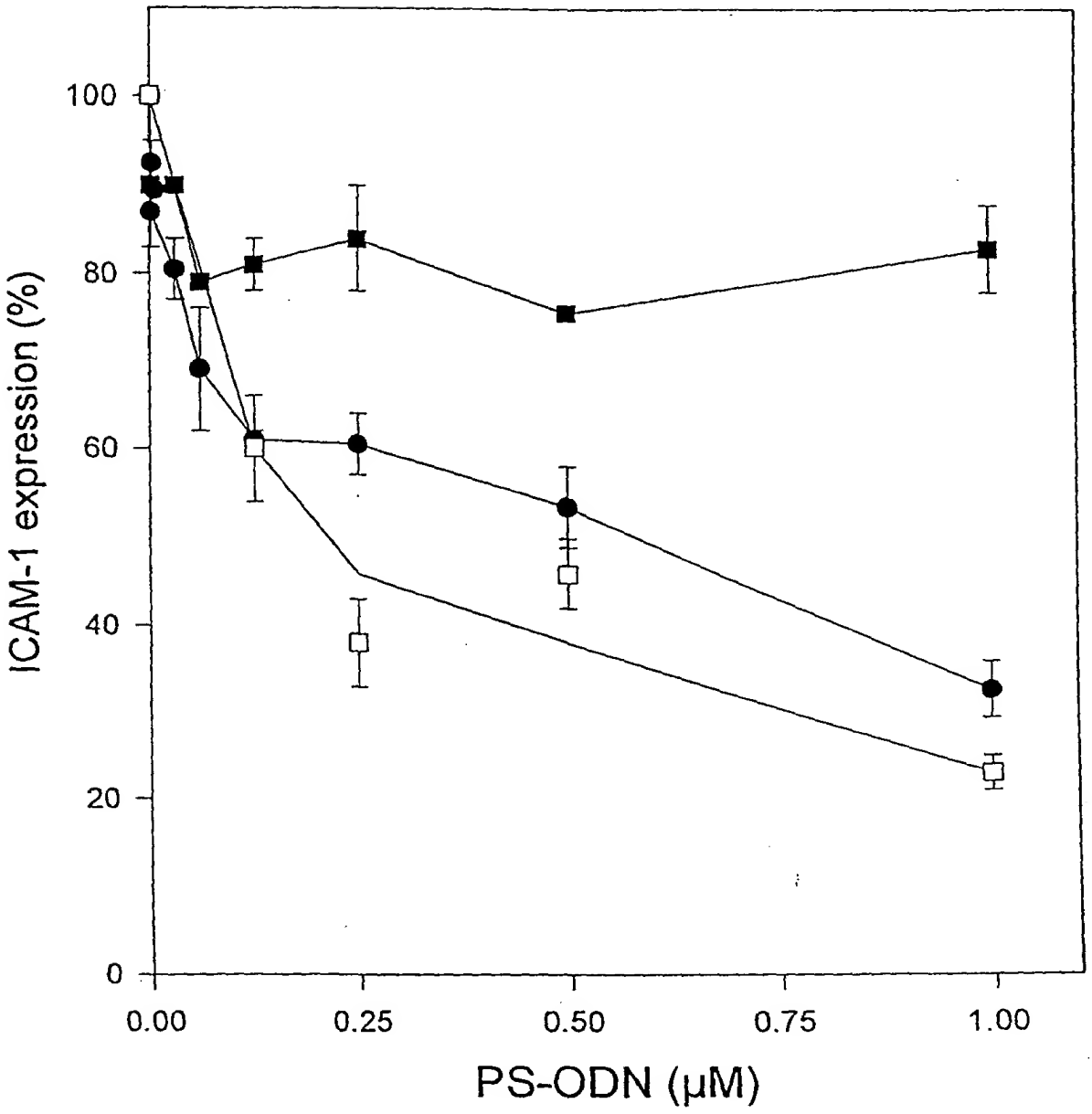

Figure 2

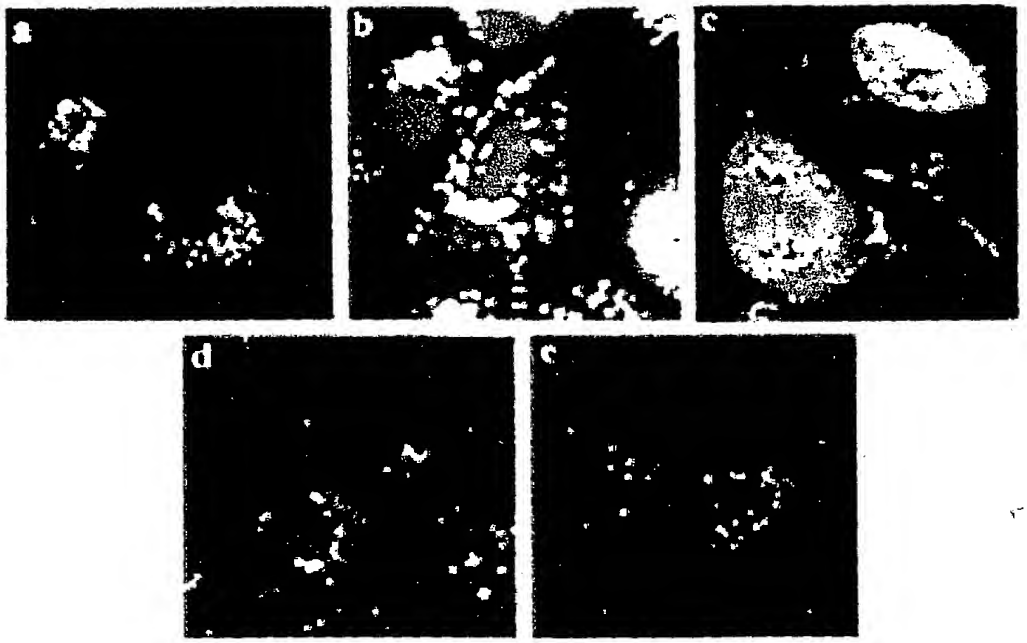

Figure 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/08980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/11 A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 22610 A (IDM IMMUNO DESIGNED MOLECULES ;MIDOUX PATRICK (FR); MONSIGNY MICHE) 28 May 1998 (1998-05-28) cited in the application * see in particular the claims; and Figures 1,5 *	1-7,9-19
A	WO 98 19710 A (SCHACHT ETIENNE HONORE ;ULBRICH KAREL (CZ); SEYMOUR LEONARD CHARLE) 14 May 1998 (1998-05-14) *see p.39,l.30 - p.40; claims 1,6-8*	1-19
A	US 5 627 270 A (HATZENBUHLER NICOLE T ET AL) 6 May 1997 (1997-05-06) * see claims 1,3; col.71,l.36-38 *	1-19

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2000	17.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Isert, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 727 223 A (HISAMITSU PHARMACEUTICAL CO) 21 August 1996 (1996-08-21) * see the claims; Figures 8-10; page 7 *	1-19
A	GOTO T. ET AL: "A novel approach for gene medicine;synthetic poly-L- lysine /serine copolymer enhances bioactivity of antisense oligonucleotides." NUCLEOSIDES AND NUCLEOTIDES, (1997) 16/7-9 (1609-1615). REFS: 10 ISSN: 0732-8311 CODEN: NUNUD5, XP002108844 United States *see in particular abstract and introduction *	1-19
A	MIDOUX P ET AL: "Membrane permeabilization and efficient gene transfer by a peptide containing several histidines." BIOCONJUGATE CHEMISTRY, (1998 MAR-APR) 9 (2) 260-7. JOURNAL CODE: A1T. ISSN: 1043-1802., XP002108845 United States *see in particular the abstract*	1-19

INTERNATIONAL SEARCH REPORT

Inte. .ational application No.
PCT/EP 99/08980

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The conjugate according to claims 1-8 is not clear in view of the broad and vague definition of both the "monomeric component" and the "protonable residues". The search had to be restricted for economic reasons and was limited to the worked examples and to the general idea underlying the application.
See Articles 5 and 6 PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/08980

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9822610 A	28-05-1998	FR 2755976 A	22-05-1998
		AU 5123998 A	10-06-1998
		EP 0946744 A	06-10-1999
WO 9819710 A	14-05-1998	AU 4873997 A	29-05-1998
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(54) Title: NEW OLIGOMERIC CONJUGATES LIABLE TO TRANSFER BIOLOGICAL MOLECULES INTO CELLS

(57) Abstract

The invention relates to a positively charged oligomeric conjugate, containing an oligomer with a polymerization degree (PD) from 5 to 50, preferably 10 to 40 and more preferably 20, formed from monomeric components having free NH_3^+ in a number equal to or higher than 50 % of the polymerization degree. In particular, the invention provides new oligomeric conjugates of histidylated oligolysine liable to allow the transfer of oligonucleotides, peptides and oligosides into cells.

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NEW OLIGOMERIC CONJUGATES LIABLE TO TRANSFER BIOLOGICAL MOLECULES INTO CELLS

The invention relates to new oligomeric conjugates liable to favor the transfer of biological molecules such as oligonucleotides, peptides and oligosides into cells.

The introduction of such molecules into cells is of great therapeutical interest.

Antisense oligonucleotides (ODN) and triplex forming oligonucleotides (TFO) are examples of attractive putative drugs in inhibiting or regulating gene expression in tumor cells and virus-infected cells.

Peptides from tumors and viruses are also attractive molecules in stimulating or elicitating a cell defense involving cytotoxic T lymphocytes against tumor and viruses after presentation by antigen presenting cells (APCs) such as dentrictic cells, macrophages and B cells.

But to be effective these molecules must reach their target in the right intracellular compartments which are either the cytosol or the nucleus.

Until now, studies of the intracellular location of oligonucleotides point out that in the majority of cells, most of the oligonucleotides are confined inside vesicles once taken up and only a small amount succeeds in reaching their RNA and DNA targets in the cytosol and in the cell nucleus.

Because once in the cytosol, the oligonucleotides penetrate rapidly into the nucleus, the enhancement of the delivery of oligonucleotides into the cytosol upon cell uptake, is expected to increase their biological activity.

Oligonucleotide encapsulation into liposomes increases their delivery into the cytosol but efficiency is drastically reduced in the presence of serum.

Antigenic peptides can bind to MHC Class I molecules and be presented at the cell surface of APCs, upon their processing via proteasomes located in the cytosol.

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Usually, peptides are taken up by APCs inside vesicles and delivered to lysosomes where they are either degraded or processed for an MHC Class II molecules presentation. Restricted MHC Class I molecules presentation by APCs requires that peptides must be introduced into the cytosol.

In French Patent n° 9613990 and PCT/FR97/02022, it has been shown that histidylated polylysine complexed with a nucleic acid is a system for cell transfection. The nucleic acid has a 10^6 to 10^8 of molecular weight. Polylysine is substituted at least 10% advantageously from 15% to 35% with molecules inducing membrane destabilization at acidic pH (mainly histidyl residues), and polylysine has a degree of polymerization of 15 to 900, particularly 200.

However, further studies have shown that the above-mentioned complex, which allows the transfection of cells by DNA, does not allow the transfer of an ODN (oligonucleotide).

One aim of the invention is to provide new positivly charged oligomeric conjugates enabling the transmembrane passage of water soluble oligomers such as oligonucleotides, peptides and oligosides into the cytosol.

An other aim of the invention is to provide new oligomeric conjugates of substituted oligolysine liable to allow the transfer of oligonucleotides, peptides and oligosides into cells.

One advantage of the invention is that the formation of a complex between new oligomeric conjugates and oligoanions such as oligonucleotides, anionic peptides or anionic oligosides, is not required to allow their transfer into the cytosol and/or the cell nucleus.

Another advantage of the invention is that, although it is not excluded, the transfer of oligoanions such as oligonucleotides, anionic peptides, anionic oligosides (i.e. sulphated, phosphorylated, succinylated or sialytaded oligosides) or a mixture thereof, does not require the formation of electrostatic complexes with the new positively charged oligomeric conjugates.

Another aim of the invention is to provide an *in vitro*, *ex vivo* and *in vivo* transfer process.

Another aim of the invention is to provide defined oligmeric compounds in which the nature of the monomeric compounds can be different from each other.

This has been achieved through the invention.

The invention, in one of its most general definitions, concerns a positively charged oligomeric conjugate containing an oligomer with a polymerization degree (PD) from 5 to 50, preferably 10 to 40 and more preferably 20, formed from monomeric components having free NH_3^+ in a number equal to or higher than 50 % of the polymerization degree,

said oligomer being as follows :

- the free NH_3^+ of the above-mentioned components are substituted in a ratio of at least 50 %, advantageously from 60 % to 95 %, particularly 80 to 90 % (this ratio being determined by nuclear magnetic resonance), by protonable residues in a weak acid medium, leading in such weak acid medium to a destabilization of cellular membranes,

- the above-mentioned protonable residues possess in addition the following properties :

→ they contain a functional group enabling them to be linked to the above-mentioned oligomer,

→ they do not correspond to a recognition signal recognized by a cellular membrane receptor,

→ they can comprise at least one free NH_3^+ group,

- the free NH_3^+ of the above-mentioned monomers can be also substituted by an uncharged residue leading to a reduction of the number of positive charges in comparison to the same oligomeric conjugate, before substitution,

- molecules constituting a recognition signal recognized by a membrane cellular receptor may be present :

→ either by substitution of some of the free NH_3^+ of the above-mentioned monomers,

→ either on some of the uncharged residues leading to a reduction of the number of charges,

→ either on some of the above-mentioned protonable residues leading to a destabilization of the cellular membranes,

→ or by substitution of the free NH_3^+ (if it is present) of the above-mentioned protonable residues leading to a destabilization of the cellular membrane,

provided that :

1) the total number of the non substituted NH_3^+ is of at least 50 % of the polymerization degree,

2) the number of monomers initially carrying free NH_3^+ is substituted in a ratio of at least 50 % of the polymerization degree by residues leading to a destabilization of the cellular membrane.

As it will result from the following, the first proviso corresponds to $m \geq i/2$ and the second proviso corresponds to $u \geq i/2$.

Said oligomeric conjugate of the invention leads to the destabilization of the membranes and allows the transfer of the above-mentioned biomolecules in the cytosol and/or the nucleus of the cells.

According to a quite unexpected effect, the oligomeric conjugates of the invention allow the transfer of ODN into the cytosol and the nucleus, without allowing the transfection of cells by DNA.

The originality of the invention lies in the fact that the oligomer of the invention must be substituted to a level of more than 50 % with molecules inducing a membrane destabilization at acidic pH (amongst such molecules are histidyl residues) because oligomeric conjugates substituted at a level lower than 50 % are cytotoxic (see table 1, hereafter). Moreover, the toxicity of oligomeric conjugates substituted at a level lower than 50 % increases with the number of free NH_3^+ of the unsubstituted lysyl residues.

For instance, for a oligomer of lysine of DP of 20 and carrying histidyl residues leading to the destabilization of the membranes, the above-said conditions can be expressed as follows :

- there must be at least 10 free NH_3^+, which come from the α-NH_3^+ and/or ε -NH_3^+,

- there are at least 10 histidyl residues, and even up to 20 histidyl residues carried by the side chain of the lysine residues which means, in the latter case, that

the minimum amount of free NH_3^+ required, i.e. "at least" 10 free NH_3^+ come only from the α-NH_3^+ functions of the histidyl residues.

The destabilization of membranes means a modification of membranes which leads either to the increase of their permeability with respect to low molecular weight (and possibly high molecular weight) molecules in solution, or the fusion with another membrane.

The membrane permeability can be measured as follows :

Cells are incubated at 37°C for 30 min in DMEM medium without serum in the presence of 0.5 mg/ml fluorescein-labelled dextran (Mw 4000) and in the absence or in the presence of an oligomeric conjugate. Cells are then washed and incubated for 30 min at 37°C in culture medium containing 10% serum. Cells are fixed for 5 min in PBS containing 4 % paraformaldehyde and the cell fluorescence localization is analysed under a fluorescent confocal microscope.

The fusion of membrane can be measured as follows :

The fusion of membrane can be measured by using liposomes according to Struck et al., (Use of resonance energy transfer to membrane fusion. 1981 Biochemistry 20: 4093-4099).

Dioleoylphosphatidylcholine (DOPC) liposomes containing N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (NBD-PE) and octtadecylrhodamine (R18) as fluorescent energy transfer donor and acceptor lipid probes, respectively, are mixed with non fluorescent liposomes and incubated in the absence or in the presence of oligomeric conjugates at various pH. Membrane fusion is evidenced by a decrease of the rhodamine fluorescence emission at 585 nm upon excitation at 470 nm, as a consequence of a decrease of the resonance energy transfer between NBD and rhodamine when the average spatial separation induced by membrane fusion increases.

The residues accounting for the destabilization of cellular membranes act through their property of being protonable in a weak acid medium.

The expression "weak acid medium" designates a medium the pH of which is lower than that of plasma or serum, i.e. a pH lower than 7.4.

Said medium can be either the extracellular medium or the lumen of intracellular compartments such as endosomes or lysosomes.

Said medium can be naturally acid or be acidified.

By way of example, the pH of said medium can be in the range of about 5 to about 7, in particular 5.5 to 6.5.

Transfer of the above-mentioned biomolecules into the cytosol and/or the cell nucleus, requires that both the oligomeric conjugate leading to the membrane destabilization and the above-mentioned biomolecules are present in the said medium.

In the present invention, the oligomeric conjugates and the above-mentioned biomolecules can be free or complexed.

Said positively charged oligomeric conjugate of the invention is liable to form a complex with at least one negatively charged oligoanion, the association between the oligoanion and the oligomeric conjugate being electrostatic in nature.

The expression according to which the protonable residues do not correspond to a recognition signal recognized by a cellular membrane receptor means that these residues are not used as ligands.

A molecule or a molecular complex is active as a recognition signal when it can be selectively recognized by a receptor, that is to say plays the role of a ligand, of an agonist, or of an antagonist. By recognition signal recognized by a cellular membrane receptor, one designates a ligand (molecule or molecular complexes) liable to be selectively recognized by said receptor.

According to an advantageous embodiment, the invention relates to a oligomeric conjugate, wherein the protonable residues leading to a destabilization of cellular membranes, present the additional properties :

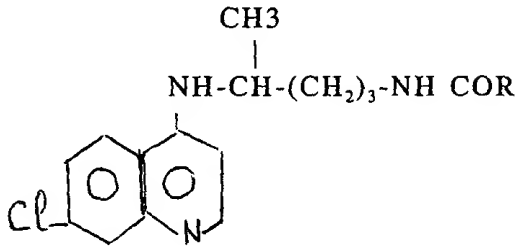
- they are weak bases, the pK of which in aqueous medium is lower than 8, so that a proportion higher than 50 % of these bases linked to a cationic oligomer is not protonated at pH 7.4.

According to another advantageous embodiment, in the oligomeric conjugate of the invention, the protonable residues leading to a destabilization of cellular membranes, present the additional properties :

- they belong to the group of compounds comprising an imidazole ring,

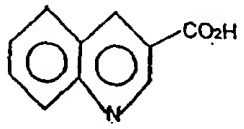
- they belong to the group of quinolins,
- they belong to the group of pterins,
- they belong to the group of pyridins.

An example of quinolin is represented by the following formulae :



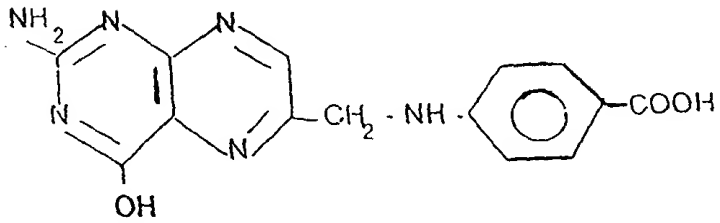
wherein R = $(CH_2)_nCO_2H$, in which n varies from 1 to 10, preferably from 1 to 3.

Another example of quinolin is :



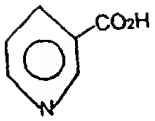
(3-quinoline carboxylic acid)

An example of pterin is represented by the following formula :

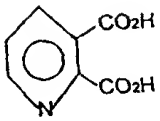


(pteroic acid)

Examples of pyridins are represented by the following formulae :



(nicotinic acid)



(quinolinic acid)

According to an advantageous embodiment, in the oligomeric conjugate of the invention, the protonable residues leading to a destabilization of the cellular membranes are :

- alkylimidazoles in which the alkyl radical comprises from 1 to 10, particularly from 2 to 6 carbon atoms, and in which only one of the nitrogen atoms of the imidazole ring is substituted.

According to an advantageous embodiment, in the oligomeric conjugate of the invention, the protonable residues leading to a destabilization of cellular membranes are chosen from :

histidine, 4-carboxymethyl-imidazole,
3-(1-methyl-imidazol-4yl)-alanine, 3-(3-methyl-imidazol-4yl)-alanine,
2-carboxy-imidazole, histamine, 3-imidazol-4yl)-L-lactic acid,
2-(1-methyl-imidazol-4yl)ethylamine, 2-(3-metyl-limidazol-4yl)ethylamine,
β-alanyl-histidine-(carnosine), 7-chloro-4(amino-1-methylbutylamino)-quinoline,

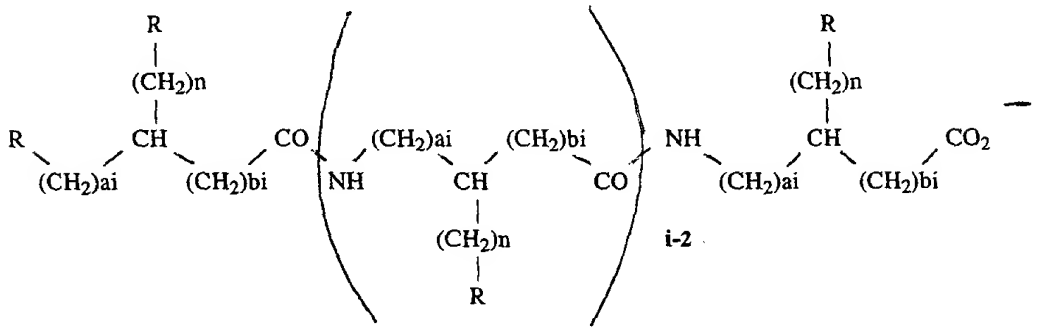
N^4-(7-chloro-4-quinolinyl)-1,4-pentanediamine,

8-(4-amino-1-methylbutylamino)-6-methoxy-quinoline (primaquine),

N^4-(6-methoxy-8-quinolinyl)1,4-pentanediamine, quininic acid,

quinoline carboxylic acid, pteroïc acid, nicotinic acid, quinolinic acid.

According to another advantageous embodiment, the oligomeric conjugate of the invention contains an oligomer of the following formula :



wherein

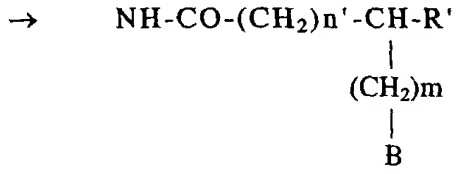
*ai is an integer varying from 0 to 10,

* bi is an integer varying from 0 to 10,

* i = degree of polymerization from 5 to 50, and particularly 10 to 40, and preferably 20,

* n = is an integer varying from 1 to 6, and preferably 4,

* R represents in a ratio of 50 % to 100 % (corresponding to a number u)



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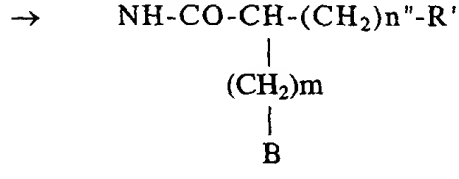

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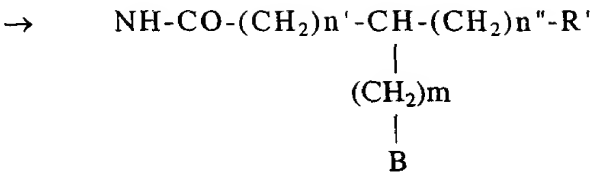


m is an integer varying from 1 to 6,

n' is an integer varying from 0 to 6,

n" is an integer varying from 0 to 6,

B is a weak base as defined above,

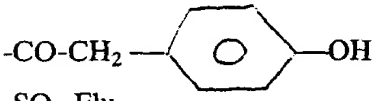
R' represents NH_3^+ (corresponding to a number p),

or NH (corresponding to a number q) substituted by

$-CO-CH_3$

$-CO-(CHOH)rH$ r being from 1 to 15 preferably 2 to 7

$-CO-(CH_2)s-(CHOH)rH$ r being from 1 to 15 preferably 1 to 7 and s being from 1 to 6 preferably 4



$-SO_2-Flu$

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

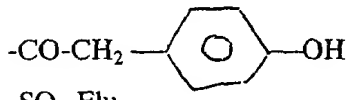
* R represents in a ratio of 0 % to 50 % (corresponding to $f : 0 < f \leq u$)

- NH_3^+ (corresponding to a number j),
- NH (corresponding to a number k), substituted by

$-CO-CH_3$

$-CO-(CHOH)rH$ r being from 1 to 15 preferably 1 to 7

$-CO-(CH_2)s-(CHOH)rH$ r being from 1 to 15 preferably 1 to 7 and s being from 1 to 6 preferably 4

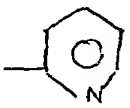


$-SO_2$-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

- H (corresponding to a number h)
- (CH_2) nH, n being an integer from 1 to 6 (corresponding to a number h)
- (CH_2) n-OH n being an integer from 1 to 6 (corresponding to a number h)
- (CH_2) n-SA' A' = H , CH_3 or 

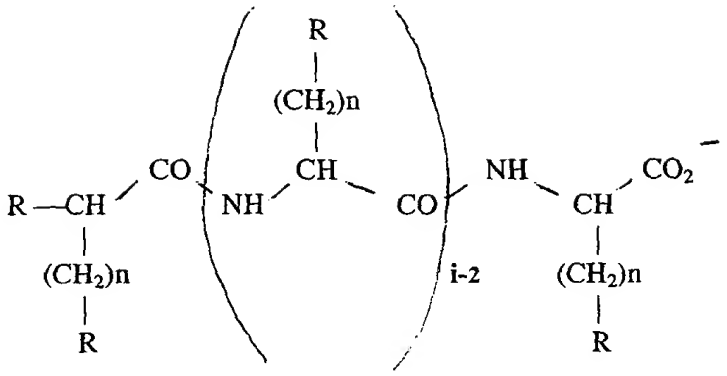
n being integer from 1 to 6 (corresponding to a number h)

with
- i = u + j + k + h
- total number of α NH_3^+ = p = u-q
- total number of ω NH_3^+ = j = f-(k + h)
- total number of NH_3^+ = m = p + j + 1

with the proviso that :

1) $u \geq i/2$
2) $m \geq i/2$

According to another advantageous embodiment, the oligomeric conjugate of the invention contains an oligomer of the following formula :



wherein

* i = degree of polymerization from 5 to 50, and particularly 10 to 40, and preferably 20,

* n = is an integer varying from 1 to 6, and preferably 4,

* R represents in a ratio of 50 % to 100 % (corresponding to u)

→ NH-CO-(CH$_2$)n'-CH-R'
|
(CH$_2$)m
|
B or

→ NH-CO-CH-(CH$_2$)n"-R'
|
(CH$_2$)m
|
B or

→ NH-CO-(CH$_2$)n'-CH-(CH$_2$)n"-R'
|
(CH$_2$)m
|
B

m is an integer varying from 1 to 6,

n' is an integer varying from 0 to 6,

n" is an integer varying from 0 to 6,

B is a weak base as defined above,

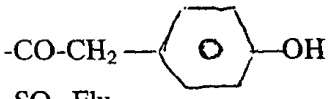
R' represents NH_3^+ (corresponding to a number p),

or NH (corresponding to a number q) substituted by

-CO-CH_3

-CO-(CHOH)rH — r being from 1 to 15 preferably 1 to 7

-CO-(CH_2)s-(CHOH)rH — r being from 1 to 15 preferably 1 to 7 and s being from 1 to 6 and preferably 4



-SO_2-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

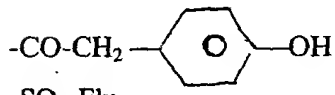
* R represents in a ratio of 0 % to 50 % (corresponding to f : $0 < f \leq 1$)

- NH_3^+ (corresponding to a number j),
- NH (corresponding to a number k), substituted by

-CO-CH_3

-CO-(CHOH)rH — r being from 1 to 15 preferably 1 to 7

-CO-(CH_2)s-(CHOH)rH — r and s being from 1 to 15 preferably 1 to 7 and s being from 1 to 6 and preferably 4

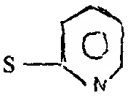


-SO_2-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

- H (corresponding to a number h)
- $(CH_2)_nH$, n being an integer from 1 to 6 (corresponding to a number h)
- $(CH_2)_n$-OH n being an integer from 1 to 6 (corresponding to a number h)
- $(CH_2)_n$-SA' A' = H , CH_3 or 

n being integer from 1 to- 6 (corresponding to a number h)

with
- i = u + j + k + h
- total number of α NH_3^+ = p = u-q
- total number of ω NH_3^+ = j = f-(k + h)
- total number of NH_3^+ = m = p + j + 1

with the proviso that :

1) $u \geq i/2$
2) $m \geq i/2$.

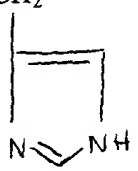
According to another advantageous embodiment, the oligomeric conjugate of the invention contains an oligomer of the formula above defined, wherein

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

n' = n" = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
CH_2

(f) R = NH^+_3
u = 12
j = 7



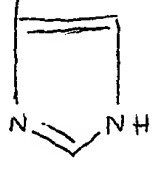
or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
(CH_2)m
B

wherein

n' = n" = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
CH_2

(f) R = NH^+_3
u = 16
j = 3

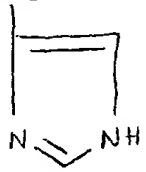


or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

n' = n" = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
|
CH_2



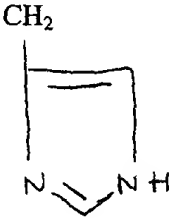
(f) R = NH^+_3
u = 19
j = 0

or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

n' = n" = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
|
CH_2



(f) R = $CO-CH_3$
u = 11
k = 8

or

i = 19 n = 4 (l) R= NH-CO-$(CH_2)n'$ -CH-$(CH_2)n''$-R'
|
$(CH_2)m$
|
B

wherein

n' = n'' = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
|
CH_2

(f) R = $CO-CH_3$
u = 15
k = 4

or

i = 19 n = 4 (u) R= NH-CO-$(CH_2)n'$ -CH-$(CH_2)n''$-R'
|
$(CH_2)m$
|
B

wherein

n' = n'' = 0
R' = NH^+_3
m = 1
B = imidazole
R = NH-CO-CH- NH_3^+
|
CH_2

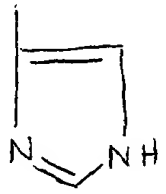
(f) R = $CO-(CHOH)rH$
r = 5
u = 12
k = 3

or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

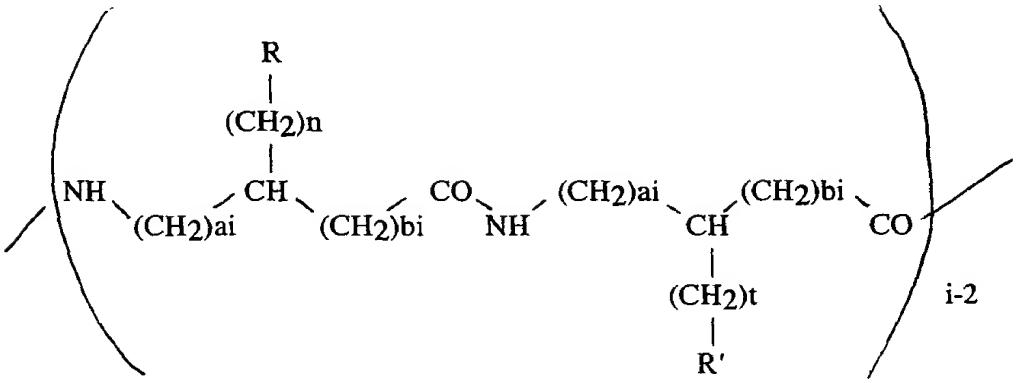
wherein

n' = n" = 0
(q) R' = NH-CO-CH_3
m = 1
B = imidazole
R = NH-CO-CH- NH-CO-CH_3
|
CH_2



(f) R = NH^+_3
u = 16
f = 4
k = 3

An example of mixed oligomer is the following :

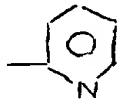


i-2

wherein

n = 4

R' = H, CH_3, $CH(CH_3)_2$, CH_2,-$CH(CH_3)_2$
$CH(CH_3)$-CH_2-CH_3, CH_2OH,
CHOH- CH_3, CH_2-CH_2-S- CH_3
(CH_2)n H, (CH_2)n-OH, (CH_2)n-SA'

A' = H, CH_3 or 

R = NH-CO-(CH_2)n' -CH-(CH_2)n" -R"
|
(CH_2)m
|
B

t varies from 1 to 6

As an example, when, in the above formula n = 4, ai = bi = 0, t = 1, R' = H, the monomers are lysine and valine.

The invention also relates to a composition containing a mixture of at least one oligomeric conjugate as defined above, with at least one biological molecule, such as a peptide, an oligoside or an oligonucleotide derivative, or a mixture thereof.

In the composition of the invention, the oligomeric conjugates can be associated with a biological molecule, in particular an oligoanion, such as an oligonucleotide, an anionic peptide or an anionic oligoside, via electrostatic interactions.

An anionic oligoside can be a sulfated oligoside, succinylated oligoside, phosphorylated oligoside, sialylated oligoside or an oligoside containing pyruvilidenyl groups.

The invention also relates to a combined preparation containing as active substance the following individual components, in the form of a kit-of-parts :

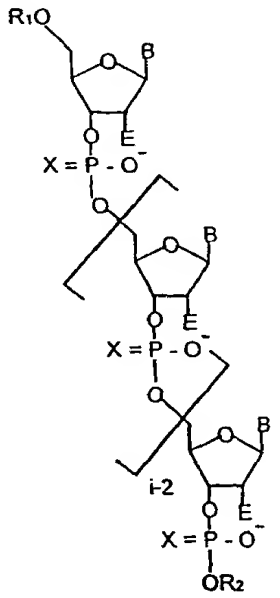
- at least an oligomeric conjugate as defined above,
- at least one biological molecule, such as a peptide, an oligoside or an oligonucleotide, or a mixture thereof,

for the simultaneous, separate or sequential use, for the *in vitro*, the *in vivo* or the *ex vivo* transfer of said biological molecules into the cytosol and/or the cell nucleus.

The invention also relates to a complex between at least one oligoanion which can be an anionic peptide, an anionic oligoside or an oligonucleotide or a mixture thereof, a mixture of at least one non negatively charged biological molecule and of at least one oligoanion, and at least one positively charged oligomeric conjugate as defined above, the association between the oligoanion and the oligomeric conjugate being electrostatic in nature.

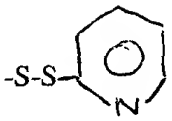
According to another advantageous embodiment, the biological molecule is chosen among oligonucleotides, peptides, oligosaccharides or a mixture thereof.

An oligonucleotide used in the invention can be of the following formula :



wherein *i* varies from 10 to 30, X represents O or S, B is a nucleic base U, A, T, G, C or a modified form such as a biotinyl or fluorescent labelled base which can be in α or β anomeric position, R1 and R2 represent independently from

each other H, OH, (CH_2)n-A, $[(CH_2)_2\text{-}O]_n\text{-}CH_2\text{-}CH_2\text{-}A$, A being H, OH, NH_2, COOH,



, n being an integer from 1 to 6,

E represents H, OH, OCH_3, OCH_2CH_3, $O(CH_2)_2CH_3$, $O(CH_2)_3CH_3$, $O(CH_2)_4CH_3$, $O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_3$

As example of oligonuclotides, one may cite the following :

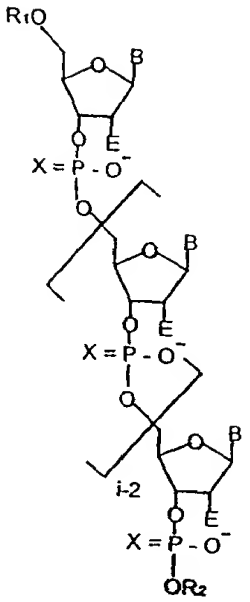
GEM 91
phosphorothioate (X = S) oligonucleotide i = 25
CTC TCG CAC CCA TCT CTC TCC TTC T
complementary to the AUG initation site of gag HIV-1 gene

ISIS 1939
phosphorothioate (X = S) oligonucleotide i = 19
CCC CCA CCA CTT CCC CTC T
complementary to the 3' non coding region of ICAM-1 mRNA.

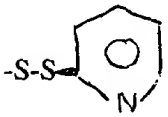
A mixture of an oligonucleotide and a peptide is defined as an oligonucleotide linked to a peptide, and a mixture of an oligonucleotide and an oligoside is defined as an oligonucleotide linked to an oligoside.

A mixture of an oligoside and a peptide is defined as an oligoside linked to a peptide.

A mixture of an oligonucleotide and a peptide or a mixture of an oligonucleotide and an oligoside used in the invention can have the following formulae :



wherein *i* varies from 10 to 30, X represents O or S, B is a nucleic base U, A, T, G, C or a modified form such as a biotinyl or fluorescent labelled base which can be in α or β anomeric position, R1 and R2 represent independently from each other H, OH, (CH_2)**n**-A, $[(CH_2)_2\text{-O}]_n\text{-}CH_2\text{-}CH_2\text{–A}$, A being H, OH, NH_2, COOH,



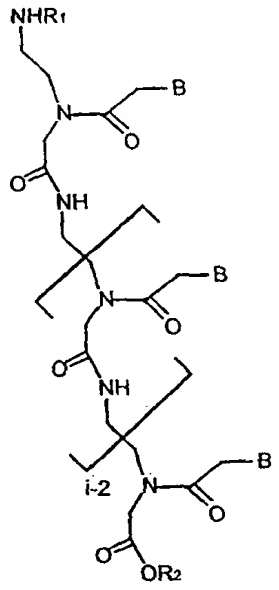
, n being an integer from 1 to 6,

or a peptide or an oligoside,

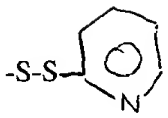
E represents H, OH, OCH_3, OCH_2CH_3, $O(CH_2)_2CH_3$, $O(CH_2)_3CH_3$, $O(CH_2)_4CH_3$, $O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_3$

When R1 and/or R2 represent a peptide, the mixed oligoanion is a peptido-oligonucleotide, and when R1 and/or R2 represent an oligoside, the mixed oligoanion represents a glyco-oligonucleotide.

An example of oligonucleotide is a peptide nucleic acid (PNA) represented by the following formula :



wherein - R1 and R2 represent independently from each other H, OH, $(CH_2)n$-A, $[(CH_2)_2\text{-}O]_n\text{-}CH_2\text{-}CH_2\text{-}A$, A being H, OH, NH_2, COOH,



, n being an integer from 1 to 6,

- i is an integer varying from 10 to 30.

In the composition of the invention, the oligonucleotide can be simple or double stranded, or can form a triplex (three strands) or a quadruplex (4 strands).

The invention also relates to the use of an oligomer conjugate as defined above, for the intracellular transfer of biological molecules into the cytosol or/and into the cell nucleus *in vitro*, *ex vivo* or *in vivo*.

The invention also relates to the use of an oligomeric conjugate as defined above or of a composition as defined above, or of a combined preparation as defined above, for the intracellular *in vitro*, *ex vivo* or *in vivo* transfer of a peptide, an oligoside or an oligonucleotide, or a mixture thereof, into the cytosol or/and in to the cell nucleus of cells.

The invention also relates to the use of an oligomeric conjugate as defined above or of a composition as defined above, or of a combined preparation as defined above, wherein the cells are chosen among muscular, epithelial, endothelial, or myeloid cells such as monocytes, macrophages, fibroblasts, leukocytes and granulocytes, osteoblasts, as well as dendritic, stem, neuronal, or dermal cells.

The invention also relates to a method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of an oligonucleotide, wherein an oligonucleotide and an oligomeric conjugate as defined above, or of a composition as defined above, or of a combined preparation as defined above, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is :

- transfer of an antisense oligonucleotide in the cytosol and/or the cell nucleus where it binds and blocks the complementary mRNA sequence,

- transfer of an oligonucleotide as activator into the cytosol where it depresses or activates a second messenger in the cytosol, or the corresponding gene in the nucleus,

- transfer into the cytosol and/or the cell nucleus of oligonucleotides corresponding to a repetitive bacterial type DNA sequence with stimulating or immunodepressive activity.

The transfer of an antisense oligonucleotide in the cytosol where it binds to the complementary mRNA sequence and blocks its traduction leading to inhibition of the synthesis of the gene product, can be carried out as described hereafter in the legends of Figures 1, 2 and 3.

The invention also relates to a method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of an oligonucleotide, wherein an oligonucleotide and an oligomeric conjugate as defined above, or of a composition as defined above, or of a combined preparation as defined above, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is :

- transfer into the cytosol and/or the cell nucleus of RNA or DNA oligonucleotide acting as decoys which inhibit gene expression by blocking the binding of regulatory factors to the authentic DNA region such as short RNA oligonucleotides corresponding to the HIV-TAR sequence inhibiting HIV expression and replication by blocking the binding of the HIV regulatory protein at Tat to the TAR region,

- transfer into the cytosol and/or the cell nucleus of ribozymes (RNA oligonucleotides) which inhibit gene expression by cleaving the mRNA.

The transfer into the nucleus of an oligonucleotide (triplex forming ODN, TFO) where it binds to target DNA at oligopurine sites where they form a triple-helical structure, leading to the inhibition of the gene expression, can be processed as hereafter described.

As an example of the specific TFO is an oligonucleotide 5'-$A_4GA_4G_6A$-3' directed against the polypurine track (PPT) in the NEF-HIV-1 gene.

The transfer of the oligonucleotide as activator of the immune response can be carried out as follows :

As an example the double strand RNA polyinosinic-polycytidylic acid (poly(I:C) for the increase of the tumoricidal activity of macrophages or for the stimulation of natural killer lymphocyte cytotoxicity.

The transfer of double stranded polynucleotide as activator of the immune response can be illustrated by the following :

Polyinosinic-polycytidylic acid (poly(I:C)) is known to increase the tumoricidal activity of macrophages *via* a TNF-α mediated cytolysis.

Thioglycolate-elicited peritoneal macrophages (2.5×10^5) are plated in 16 mm diameter well multiwell plates in serum free RPMI medium. Upon 2 h incubation at 37°C, non-adherent cells are discarded. Adherent cells are cultured in 0.5 ml of serum free RPMI medium in the absence or the presence of polyI:C and in the absence or the presence of histidylated oligolysine. Culture supernatants are collected after 24 h incubation at 37°C. Before testing, supernatants are rendered cell-free by centrifugation at 2000 g for 10 minutes. The cytotoxic activity of macrophage culture supernatants is determined by using L929 cells pretreated for 2 h at 37°C with 2 μg/ml actinomycin D. Actinomycin D pretreated L929 cells are seeded in 96-wells microplates (4×10^4 cells in 0.05 ml serum free RPMI medium per well). After 3-4 h at 37°C, diluted supernatants from stimulated macrophages are added (0.05 ml) and the cells are incubated at 37°C for 18-20 h. Microplates are washed with PBS and the percentage of cell lysis is determined after staining the cells with 0.05 ml of crystal violet (0.2% in 2 % ethanol). The plates are washed with tap water and the dye is solubilized by adding 0.06 ml per well of 0.3 % sodium dodecyl sulfate. Absorbance of each well is read at 570 nm. The % cytotoxicity is calculated according to $(A_{NS} - A_S)/A_{NS} \times 100$ where A_{NS} and A_S are the absorbances of wells containing target cells incubated with supernatant dilutions of non stimulated and stimulated macrophages, respectively.

The invention also relates to a method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of peptide, wherein a peptide and an oligomeric conjugate as defined above, or of a composition as defined above, or of a combined preparation as defined above, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is a transfer of said peptide into the cytosol.

The transfer of a peptide can be illustrated by the following :

A) Cells are incubated for 4 h at 37°C with 1 μM fluorescein-labelled peptide (*F-S*-CGEEDTSEKDEL) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.

B) Dendritic cells are incubated for 4 h at 37°C with 1 μM c-myc epitope peptide (SMEQKLISEEDLNFELDEA) in the absence or in the presence of histidylated oligolysine. Cells are fixed with 2 % of p-formaldehyde in the presence of 0.5 % saponine, washed and then incubated for 1 h with anti c-myc epitope monoclonal antibody (9E10) in PBS containing containing 10 mg/ml BSA and 0.1% saponin. Cells are washed and further incubated for 1 h in the presence of fluorescein-labelled anti-mouse IgG F(ab)' fragments in PBS containing containing 10 mg/ml BSA and 0.1% saponin. Cells are washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope.

The fixation of a peptide to intracellular cofactor can be carried as described in the following example:

An oligopeptide corresponding to a prostate specific antigen (PSA) epitope mixed to the oligomeric conjugate is transferred into the cytoplasm of macrophages.

The oligopeptide is fixed there to heat shock protein s (HSP90, HSP70) to form HSP–peptide complexes which are then re-expressed at the surface of macrophages. This complex formed with the HSP cofactor stimulate macrophages and enhance the immune response to the PSA antigen.

The invention also relates to a method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of peptide, wherein a peptide and an oligomeric conjugate as defined

above, or a composition as defined above, or a combined preparation as defined above, in particular wherein the peptide is an antigenic peptide, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is a transfer of said antigenic peptide in the cytosol of antigen presenting cells (macrophages, dendritic cells and B cells) where they are processed in proteosomes in order to bind to MHCI molecules, allowing the presentation of the antigenic epitope fixed on MHCI.

In vitro evaluation of antigen presentation can be illustrated by the following:

Dentritic cells were incubated for 4 h at 37°C with the nonadecapeptide (185-203) from the C-terminal part of the HIV-1 Nef protein containing the nonapeptide (190-198) (AFHHVAREL) in the absence or in the presence of an histidylated oligolysine. Cells are washed and further incubated for 24 h at 37°C in the absence of peptide and histidylated oligolysine. MHC class I presentation of peptide antigen was evaluated by Cr^{51} cytotoxic assay by using a CTL clone sensible to the peptide. DCs were labelled with Cr^{51} (target cells : T) and then incubated at 37°C for 4 h in the presence of the CTL clone (effector cells : E) at E/T ratios ranged from 1 to 100. The supernatants are collected and the radioactivity in the supernatant was recorded. The % of specific Cr^{51} release is calculated according to $(A_{NS} - A_S)/A_{NS} \times 100$ where A_{NS} and A_S are the radioactivity in supernatant dilutions of dentritic cells incubated in the absence and the presence of CTL cells, respectively.

The invention also relates to a method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of a oligoside, wherein an oligoside and an oligomeric conjugate as defined above, or a composition as defined above, or a combined preparation as defined above, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is a transfer of said oligoside into the cytosol and/or the cell nucleus.

An example of transfer of an oligoside can be illustrated by the following :

Cells are incubated for 4 h at 37°C with 0.5 mg/ml fluoresein-labelled dextrans (either Mw 4000 or Mw 70000) in the absence or in the presence of an oligomeric conjugate. Cells are washed with PBS, fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v)

containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope (Nikon, Tokyo, Japan) and a planapo objective (numerical aperture 1.4).

An example of transfer of a negatively charged oligoside can be illustrated by the following :

Cells are incubated for 4 h at 37°C with 0.5 mg/ml fluoresein-labelled polyanionic dextrans (either Mw 3000 or Mw 70000) in the absence or in the presence of an oligomeric conjugate. Cells are washed with PBS, fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells are analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope (Nikon, Tokyo, Japan) and a planapo objective (numerical aperture 1.4).

The fixation of an oligoside to intracellular cofactor can be carried as described in the following example:

An oligoanion with silicated saccharidic components complexed according to the invention is transported into the cytoplasm of human cells where it binds to intracellular cofactors or second messengers such as NF kappa B. This binding causes nuclear transfer of the cofactor which derepress or stimulates genes coding for cytokines (such IL1, TNF-α, IL-12).

This results in a marked stimulation of the human cell cultured in the presence of the complex.

The invention also relates to a pharmaceutical composition, comprising as active substance at least an oligomeric conjugate as defined above, or a composition as defined above, or a combined preparation as defined above, or in association with a pharmaceutically acceptable vehicle.

The invention also relates to the use of an oligomeric conjugate as defined above, or of a composition as defined above, or of a combined preparation as defined above, or for the preparation of a drug for use in the treatment of cancer, inflammatory or immunology diseases (such as graft rejection, allergy, auto-immunity) or infectious diseases.

The invention also relates to a kit or case containing :

- an oligomeric conjugate as defined above, substituted by a protonable residue leading in a weak acid medium to a destabilization of cellular membranes, this oligomeric conjugate being able to comprise a recognition signal, which is previously fixed or not on the above-said conjugate, said recognition signal being dependent upon the cell to target,
- at least one biological molecule to transfer,
- optionally reagents enabling the possible binding of the recognition signal on the above-said oligomeric conjugate,
- optionally reagents enabling the formation of a composition as defined above, or of a combined preparation as defined above,
- reagents enabling the transfer of the biological molecule in the cytosol and/or the cell nucleus.

LEGEND OF THE FIGURES

Inhibition of luciferase gene expression by GEM-91.

Figure 1 shows the activity of GEM-91, an antisense phosphorothioate oligonucleotide (PS-ODN) (CTC TCG CAC CCA TCT CTC TCC TTC T) complementary to the AUG initiation site of gag HIV-1 gene. The effect of histidylated oligolysines was evaluated by using pRET-Luc cells (a rabbit smooth muscle cell line). These cells produce endogenous luciferase under the control of the human phosphoglycerate kinase promoter and the luciferase gene sequence around the AUG codon was replaced by the initiator AUG codon and several downstream codons of gagHIV-1 gene. The results showed that the activity of GEM-91 (IC_{50} > 5 μM) was increased more than 10 times in the presence of 20 μM HoK2 (IC_{50} 0.25 μM). Whilst, no significant inhibition was obtained in the presence of HoK3 in which the α-NH_2 histidyl residues were acetylated, suggesting that interactions between ODN and histidylated oligolysines were involved. pRET-Luc cells, seeded onto 24-well plates (2 x10^5 cells/well), were treated for 4 h at

37°C in DMEM supplemented with 2 % FBS containing various concentrations of GEM-91, (■) in the absence of histidylated oligolysine, ( ) in the presence of 20 μM HoK2 or (○) in the presence of 20 μM HoK3. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. Then, FBS was raised to 6 % and cells were further incubated for 18 h. Luciferase gene expression was measured by recording luminescence for 4 s. The percentage of luciferase inhibition was calculated by using , [(RLU^{ODN}-RLU)/RLU] x 100 where RLU^{ODN} and RLU were the luciferase activity into cell lysates of cells incubated in the absence and in the presence of ODN, respectively. Results shown typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation.

Inhibition of TNF-α induced ICAM-1 expression by ISI 1939.

Figure 2 shows the inhibitory effect of TNF-α induced ICAM-1 expression by ISIS 1939 (CCCCCACCACTTCCCCTCT), an antisense phosphorothioate oligonucleotide (PS-ODN) targeted to the 3' non-coding region of ICAM-1 mRNA. The results showed that TNF-α induced ICAM-1 expression was inhibited by ISIS 1939 in the presence of 20 μM of histidylated oligolysines. HoK2 (IC_{50} of 0.25 μM) appeared to be more efficient than HoK1 (IC_{50} of 0.5 μM) probably because HoK2 bore less histidyl residues than HoK1 (15 *versus* 12). The inhibition was very low in the absence of histidylated oligolysines even up to 1 μM ODN (20 % inhibition). A549 cells (ATCC CCL 185, ATCC Rockville, MD) were plated onto 96-wells microtiter plates (10^4 cells /well). The day after, culture medium was removed and cells were washed. Cells were incubated at 37°C for 4 h in 100 μl DMEM serum-free medium containing ISIS 1939 ODN either in the absence (■) or in the presence of 20 μM (●) HoK1 or (□) HoK2. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. One volume of fresh medium containing 10 ng/ml TNF-α was added and cells were further incubated for 18 h. ICAM-1 expression was quantified by ELISA using anti-ICAM-1 antibodies. Cells were washed 3 times with 200 μl of PBS and fixed for 20 min at room temperature in PBS containing 20 mg/ml paraformaldehyde. Then, cells were incubated for 90 min at 37°C with anti-ICAM 1 mouse antibody (Becton

Dickinson) diluted 20 times in PBS containing 20 mg/ml BSA. Cells were washed 3 times with PBS and then incubated for 1 h at 37°C with an anti-mouse horseradish peroxydase conjugate (Becton Dickinson) diluted 2000 times in PBS containing 20 mg/ml BSA. After 3 whashes, the peroxydase activity was assessed by using 100 μl of o-phenylenediamine dihydrochloride peroxydase substrate tablet set (Sigma). After X min incubation at 37°C, the reaction was stopped by adding 25 μl of 3 N H_2SO_4 and the absorbance read at 492 nm. All calculations were made relative to untreated controls in the absence or in the presence of TNF-α. The percentage of TNF-α–induced expression of ICAM-1 was calculated as follows : $[(A_{TNF-\alpha}{}^{ODN} - A_0) / (A_{TNF-\alpha}-A_0)] \times 100$ where $A_{TNF-\alpha}{}^{ODN}$ was the absorbance of ODN treated and cytokine-induced cells, A_0 the absorbance of cells incubated without ODN and TNF-α, and $A_{TNF-\alpha}$ the absorbance of cytokine-induced cells incubated without ODN. Results shown are typical of experiments carried out in triplicate and repeated at least twice. Data are means ± standard deviation of the percentage of control ICAM-1 expression induced by TNF-α.

Effect of histidylated polymers on the intracellular location of PS-ODN.

Figure 3 shows that histidylated oligolysines induced cytosolic and nuclear delivery of ODN. A549 cells incubated for 4 h at 37°C with 0.125 μM *F*-PS-ODN in the absence of histidylated oligolysine exhibited a faint vesicular staining (Fig 3-a). In the presence of either HoK1 (Fig 3-b) or HoK2 (Fig 3-c), the fluorescent staining was more intense in agreement with the flow cytometry analysis. HoK2 and HoK3 are histidylated oligolysines prepared as described in the following text. In addition, the vesicles appeared bigger and the cytosol and the nucleus were also labelled while vesicles were smaller and neither the cytosol and the nuclear were staining in the absence of histidylated oligolysine (Fig 3-a). The cytosolic and nuclear staining was greater in the presence of HoK2 than in the presence of HoK1, probably because HoK2 contained more histidyl residues than HoK1 (15 *versus* 12). In contrast, the cell associated fluorescence was low in the presence of HoK3 suggesting that interactions between ODN and HoK1 and HoK2 might favor the ODN uptake (Fig 3-c). Unsubstituted oligolysine had no effect on the ODN uptake and ODN accumulation (Fig 3-d). A549 cells (ATCC CCL 185,

ATCC Rockville, MD) were seeded onto sterile coverslips in 20-mm wells (2 x 10^5 cells/ well) and allowed to adhere. Cells were incubated in the presence of 0.125 μM fluorescein-labelled PS-ODN for 4 h at 37°C (a) in the absence or in the presence of 20 μM (b) HoK1, (c) HoK2, (d) HoK3 or (e) Plk (Oligolysine containing 19 lysyl residues and non substituted by hisditine used as control). Cells were fixed with 2 % of p-formaldehyde, washed and mounted on slides in a PBS/glycerol mixture (1:1 v/v) containing 10 mg/ml DABCO (1,4 diazobicyclo-(2,2,2)-octane) as antifading agent. Cells were analyzed with a confocal microscope imaging system (MRC-1024, Bio-Rad) equipped with a Nikon Optiphot epifluorescence microscope (Nikon, Tokyo, Japan) and a planapo objective (numerical apErture 1.4).

EXAMPLES

Preparation of histidylated oligolysines :

Oligolysine (Poly-L-lysine, HBr ; average molecular weight of 3950 ; average degree of polymerization of 19) (Bachem Feinchemikalien, Bubendorf, Switzerland) (1 g in 200 ml H_2O) was passed through an anion exchange column (Dowex 2 x 8, ^-OH form, 20-50 mesh) in order to remove bromide ions. The eluate was neutralized with a 10 % *p*-toluene sulfonic acid solution in water and freezed-dried.

Example 1 : Preparation of HoK1

Oligolysine *p*-toluene sulfonate salt (50 mg ; 8.6 μmol) in 2 ml dimethylsulfoxide (Aldrich, Strasbourg, France) in the presence of diisopropylethylamine (50 μl ; 344 μmol) (Aldrich) was reacted for 20 h at 20°C with (Boc)His(Boc)-OH (64 mg ; 146 μmol) (Novabiochem, Bad Soden, Germany) in the presence of benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexafluorophosphate (BOP) (Richelieu Biotechnologies, Saint Hyacinthe, Canada) (159mg ; 358 μmol). The residual ε-amino groups of oligolysine was then substituted with gluconoyl residues (GlcA) : δ-gluconolactone (86mg ; 48 μmol)

(Aldrich) and diisopropylethylamine (134 µl ; 921 µmol) were added and the solution was stirred for 20h at 20°C. The *N*-protecting Boc groups were removed by acidic treatment by adding 10 volumes of a H_2O/trifluoroacetic acid mixture (1:1 ; v/v) for 24h at 20°C. Water and trifluoroacetic acid were removed under reduced pressure. HoK1 was precipitated by adding 10 volumes of isopropanol and spun down by centrifugation (1800g for 15 min). The pellet was washed with isopropanol, collected by centrifugation (1800g for 15 min), solubilized in distilled water and freezed-dried. The average number of histidyl residues bound per oligolysine molecule was determined by ^{1}H-NMR spectroscopy at 300 MHz in D_2O according to : $x = 6.(h_{8.7} / h_{Lys})$. DP, where $h_{8.7}$ was the value of the integration of the signal at 8.7 ppm corresponding to the proton (1H C_{12}) of histidyl residues, h_{Lys} that in the range from 1.3 to 1.9 ppm corresponding to the 6 methylene protons (C_3, C_4 and C_5) of lysyl residues and DP the degree of polymerization of oligolysine. The number of histidyl residues bound per oligolysine molecule was 12. The average number of gluconoyl residues bound per oligolysine molecule was determined by ^{1}H-NMR spectroscopy from : $x = 3/2.(h_G / h_{Lys})$. DP, where h_G was the value of the integration in the range 3.6 to 3.9 ppm of the 4 protons (1H C_{10}, 1H C_{11} and 2H C_{12}) of gluconoyl residues, h_{Lys} that in the range of 1.3 to 1.9 ppm of the 6 methylene protons (C_3, C_4 and C_5) of lysyl residues and DP the degree of polymerization of pLK. The number of gluconoyl residues bound per oligolysine molecule was 3. The number of free ε-amino groups per oligolysine molecule was 4.

Example 2 : Preparation of HoK2

Oligolysine *p*-toluene sulfonate salt (80 mg ; 13.7 µmol) in 3 ml dimethylsulfoxide in the presence of diisopropylethylamine (90 µl ; 620 µmol) was reacted for 6 h at 20°C with the N-hydroxysuccinimidyl derivative of (Boc)His(Boc)-OH (92 mg ; 204 µmol) (Bachem Feinchemikalien). The residual ε-amino groups of oligolysine was then acetylated (Ac) : acetic anhydride (13 µl ; 109 µmol) (Aldrich) and diisopropylethylamine (5 µl ; 35 µmol) were added and the solution was stirred for 30 min at 20°C. The *N*-protecting Boc groups were removed by acidic treatment by adding 10 volumes of a

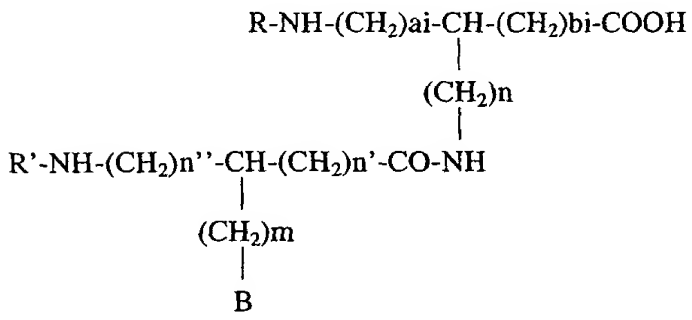
H_2O/trifluoroacetic acid mixture (1 : 1 ; v/v) for 2 h at 20°C. Water and trifluoroacetic acid were removed under reduced pressure. HoK2 was precipitated by adding 10 volumes of isopropanol and spun down by centrifugation (1800 g for 15 min). The pellet was washed with isopropanol, collected by centrifugation (1800 g for 15 min), solubilized in distilled water and freezed-dried. The average number of histidyl residues bound per oligolysine molecule was determined by ^{1}H-NMR spectroscopy at 300 MHz in D_2O according to : $x = 6 . (h_{8.7} / h_{Lys})$. DP, where $h_{8.7}$ was the value of the integration of the signal at 8.7 ppm corresponding to the proton (1H C_{12}) of histidyl residues, h_{Lys} that in the range from 1.3 to 1.9 ppm corresponding to the 6 methylene protons (C_3, C_4 and C_5) of lysyl residues and DP the degree of polymerization of oligolysine. The number of histidyl residues bound per oligolysine molecule was 15. The average number of acetyl residues bound per oligolysine molecule was determined by ^{1}H-NMR spectroscopy from : $x = (h_A / h_{Lys})$. DP, where h_A was the value of the integration at 2.04 ppm of the 3 protons of acetyl residues, h_{Lys} that in the range of 1.3 to 1.9 ppm of the 6 methylene protons (C_3, C_4 and C_5) of lysyl residues and DP the degree of polymerization of pLK. The number of acetyl residues bound per oligolysine molecule was 3. The number of free ε-amino groups per oligolysinemolecule was 1.

Example 3 : Preparation of HoK3

Oligolysine *p*-toluene sulfonate salt (85 mg ; 14.6 μmol) in 3 ml dimethylsulfoxide in the presence of diisopropylethylamine (80 μl ; 555 μmol) was reacted for 20 h at 20°C with N-acetyl-His-OH (288 mg ; 307 μmol) (Sigma) in the presence of BOP (265 mg ; 597 μmol). The residual ε-amino groups of oligolysine was then acetylated (Ac) with acetic anhydride for 30 min at 20°C. HoK3 was precipitated by adding 10 volumes of isopropanol and spun down by centrifugation (1800 g for 15 min). The pellet was washed with isopropanol, collected by centrifugation (1800 g for 15 min), solubilized in distilled water and freezed-dried. The average number of histidyl residues bound per oligolysine molecule was determined by ^{1}H-NMR spectroscopy as describe The number of

histidyl residues bound per oligolysine molecule was 15. The number of free ε-amino groups per oligolysine molecule was 1.

Example 4 : Preparation of synthons for synthesis of oligomeric conjugates



wherein

R and R' represent aminoprotecting groups, ai is an integer varying from 0 to 6, bi is an integer varying from 0 to 6, n is an integer varying from 1 to 6, n' is an integer varying from 0 to 6, n" is an integer varying from 0 to 6, m is an integer varying from 1 to 6.

An example of synthon : the Lys(His) synthon

wherein

ai = bi = 0
n = 4
R1 = Fmoc
n' = n'' = 0
m = 1
R' = Boc
B = (NBoc)Imidazole

The N-hydroxysuccinimidyl derivative of $(Boc)_2$-His-OH [$(Boc)_2$-His-OSu] (1g ; 2.2 mmol) is coupled to Fmoc-Lys-OH (722 mg ; 2.2 mmol) for 24 h at 20°C in dimethylformamide (ml). The Lys(His) synthon is precipitated with isopropanol, collected by centrifugation, washed with ether and dried under vaccum. The Lys(His) synthon is purified by cristallization.

Example 5 :

Preparation of an histidylated oligolysine (HoK) containing 20 lysine residues and 20 histidyl residues.

A HoK containing exactly 20 lysyl residues and 20 histidyl residues can be entirely synthetised by using the above Lys (His) synthon. Briefly, 20 Lys(His) synthon are successively assembled on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons are coupled by the HBTU activation method. HoK aree cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. Crude HoK is precipitated with isopropanol and collected by centrifugation. HoK is washed three times with isopropanol, resuspended in distilled water and freezed-dried.

Example 6

Preparation of an oligomeric conjugate containing 17 lysyl residues substituted with 17 histidyl residues and 3 leucyl residues inserted any where in the lys (His) sequence

Oligomers made of exactly 17 lysyl residues substituted with 17 histidyl residues and 3 leucyl residues insert anywhere in the Lys(His) sequence can be entirely synthetised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu were coupled by the HBTU activation method. Oligomers are cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. Oligomers are precipitated with isopropanol and collected by centrifugation. Oligomers are washed three times with isopropanol, resuspended in distilled water and freezed-dried.

Example 7 : Preparation of (K(His)-KL(His)-L)$_7$

An oligomer (K(His)-K(His)-L)$_7$ can be entirely synthetised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer (K(His)-K(His)-L)$_7$ is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The polymer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.

Example 8 : Preparation of (K(His)-L-K(His))$_7$

A oligomer (K(His)-L-K(His))$_7$ can be entirely synthetised by using the above Lys (His) synthon and Fmoc Leu on a Applied Biosystems 433A synthesizer with conductimetric monitoring by using Fmoc-protected amino acids. Lys(His) synthons and Leu are coupled by the HBTU activation method. The oligomer is cleaved from the resin and side chain protecting Boc groups are removed with a trifluoroacetic acis/water mixture (50% : 50% ; v/v) for 3 h at room temperature. The oligomer is precipitated with isopropanol and collected by centrifugation. The oligomer is washed three times with isopropanol, resuspended in distilled water and freezed-dried.

Table I shows that oligolysine having a DP of either 190, 72 or 36 substituted with less that 45% of histidyl residues, do not allow the transfer of small nucleic acid, particularly oligonucleotides. Indeed, it is necessary to adapt the size of histidylated oligolysines. Conversely, small histidylated oligolysines (DP of 36 or 19) substituted by more than 50% of histidyl residues do not allow efficient gene transfer by histidylated oligolysine/plasmid complexes (Table I). In addition oligolysines substituted with less than 50% histidyl residues are cytotoxic.

Table I : comparative evaluation of gene transfer and oligonucleotide transfer by using histidylated polylysine.

DP	His (%)	DNA	ODN	Cytotoxicity (%)
190	35	100	0	24
190	45	110	0	21
72	23	87-96	0	24
36	22	61-100	0	25
36	53	0-10	20	4
19	25	15-26	0	49
19	45	9	0	40
19	60	nd	100	26
19	80	nd	100	0
19	100	nd	100	0

DP is the oligolysine degree of polymerization. DNA corresponds to transfection by using histidylated oligolysine/pCMVLUC. The transfection efficiency is scored on a 0 to 100 scale. The transfection efficiency is determined from the luciferase activity in cells measured by luminescence. ODN corresponds to cytosolic and nuclear transfer of fluorescein-labelled oligonucleotide in the presence of histidylated oligolysine, evaluated under confocal microscope. Cytotoxicity was evaluated by using the colorimetric MTT assay. (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

CLAIMS

1. Oligomeric conjugate positively charged, containing an oligomer with a polymerization degree (PD) from 5 to 50, preferably 10 to 40 and more preferably 20, formed from monomeric components having free NH_3^+ in a number equal to or higher than 50 % of the polymerization degree,

said oligomer being as follows :

- the free NH_3^+ of the above-mentioned components are substituted in a ratio of at least 50 %, advantageously from 60 % to 95 %, particularly 80 to 90 % (this ratio being determined by nuclear magnetic resonance), by protonable residues in a weak acid medium, leading in such a weak acid medium to a destabilization of cellular membranes,

- the above-mentioned protonable residues possess in addition the following properties :

→ they contain a functional group enabling them to be linked to the above-mentioned oligomer,

→ they do not correspond to a recognition signal recognized by a cellular membrane receptor,

→ they can comprise at least one free NH_3^+ group,

- the free NH_3^+ of the above-mentioned monomers can be also substituted by an uncharged residues leading to a reduction of the number of positive charges in comparison to the same oligomeric before substitution,

- molecules constituting a recognition signal recognized by a membrane cellular receptor may be present :

→ either by substitution of some of the free NH_3^+ of the above-mentioned monomers,

→ either on some of the uncharged residues leading to a reduction of the number of charges,

→ either on some of the above-mentioned protonable residues leading to a destabilization of the cellular membranes,

→ or by substitution of the free NH_3^+ (if it is present) of the above-mentioned protonable residues leading to a destabilization of the cellular membrane,

provided that :

1) the total number of the non substituted NH_3^+ functions is of at least 50 % of the polymerization degree,

2) the number of monomers initially carrying free NH_3^+ is substituted in a ratio of at least 50 % of the polymerization degree by residues leading to a destabilization of the cellular membrane.

2. Oligomeric conjugate according to claim 1, wherein the protonable residues leading to a destabilization of cellular membranes, present the additional properties :

- they are weak bases the pK of which in aqueous medium is lower than 8, so that a proportion higher than 50 % of these bases linked to a cationic oligomer is not protonated in a neutral medium of pH 7.4.

3. Oligomeric conjugate complex according to claim 1, wherein the protonable residues leading to a destabilization of cellular membranes, present the additional properties:

- they belong to the group of compounds comprising an imidazole ring,
- they belong to the group of quinolins,
- they belong to the group of pterins,
- they belong to the groupe of pyridins.

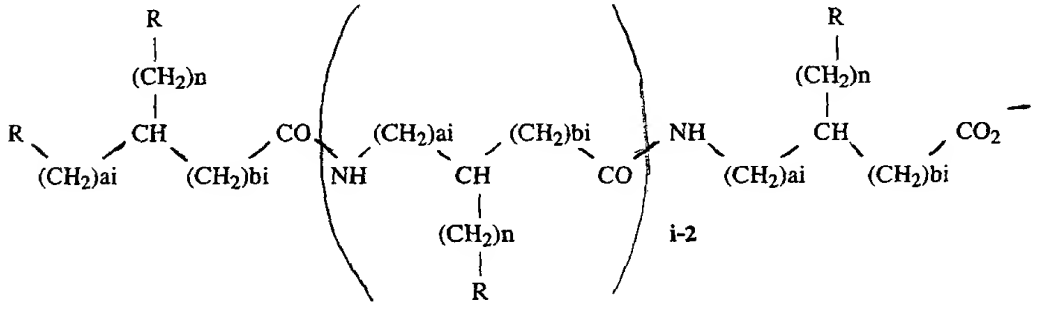
4. Oligomeric conjugate according to anyone of claims 1 to 3, wherein the protonable residues leading to a destabilization of the cellular membranes are :

- alkylimidazoles in which the alkyl radical comprises from 1 to 10, particularly from 2 to 6 carbon atoms, and in which only one of the nitrogen atoms of the imidazole ring is substituted.

5. Oligomeric conjugate according to anyone of claims 1 to 4, wherein the protonable residues leading to a destabilization of cellular membranes are chosen from :

histidine, 4-carboxymethyl-imidazole, 3-(1-methyl-imidazol-4yl)-alanine, 3-(3-methyl-imidazol-4yl)-alanine, 2-carboxy-imidazole, histamine, 3-imidazol-4yl)-L-lactic acid, 2-(1-methyl-imidazol-4yl)ethylamine, 2-(3-metyl-limidazol-4yl)ethylamine, β-alanyl-histidine-(carnosine), 7-chloro-4(amino-1-methylbutylamino)-quinoline, N^4-(7-chloro-4-quinolinyl)-1,4-pentanediamine, 8-(4-amino-1-methylbutylamino)-6-methoxy-quinoline (primaquine), N^4-(6-methoxy-8-quinolinyl)1,4-pentanediamine, quininic acid, quinoline carboxylic acid, pteroïc acid, nicotinic acid, quinolinic acid.

6. Oligomeric conjugate according to anyone of claims 1 to 5, wherein the oligomeric conjugate contains an oligomer of the following formula :



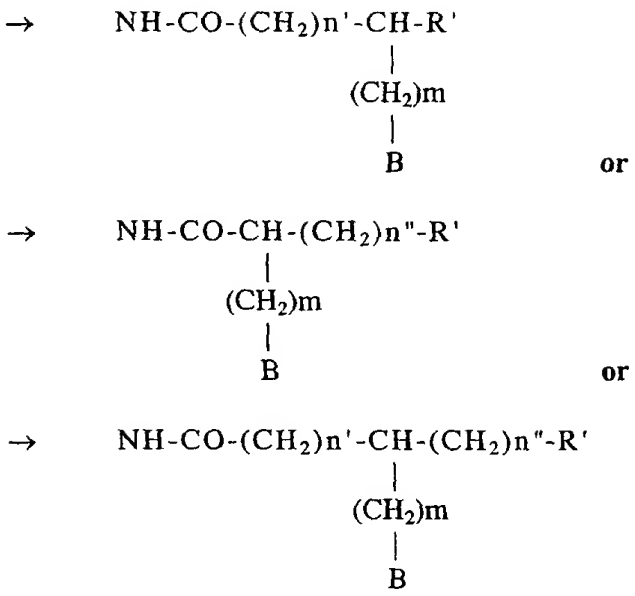
wherein * ai is an integer varying from 0 to 10,

* bi is an integer varying from 0 to 10,

* i = degree of polymerization from 5 to 50, and particularly 10 to 40, and preferably 20,

* n = is an integer varying from 1 to 6, and preferably 4,

* R represents in a ratio of 50 % to 100 % (corresponding to a number u)



or

or

m is an integer varying from 1 to 6,

n' is an integer varying from 0 to 6,

n" is an integer varying from 0 to 6,

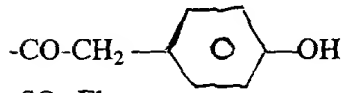
B is a weak base as defined according to anyone of claims 2 to 4,

R' represents NH_3^+ (corresponding to a number p),
or NH (corresponding to a number q) substituted by

$-CO-CH_3$

$-CO-(CHOH)rH$ r being an integer from 1 to 15, and preferably 1 to 7

$-CO-(CH_2)s-(CHOH)rH$ r being an integer from 1 to 15, and preferably 1 to 7, and s being an integer from 1 to 6, and preferably 6



$-SO_2$-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

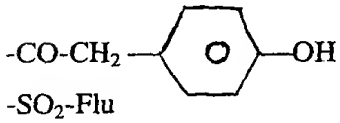
* R represents in a ratio of 0 % to 50 % (corresponding to f : $0 < f \leq u$)

- NH_3^+ (corresponding to a number j),
- NH (corresponding to a number k), substituted by

-$CO-CH_3$

-CO-(CHOH)rH r being an integer from 1 to 15, and preferably 1 to 7

-$CO-(CH_2)s-(CHOH)rH$ r being an integer from 1 to 15,and preferably 1 to 7, and s being an integer from 1 to 6, and preferably 6



-SO_2-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

- H (corresponding to a number h)
- (CH_2) nH, n being an integer from 1 to 6

(corresponding to a number h)

- (CH_2) n-OH n being an integer from 1 to 6

(corresponding to a number h)

- (CH_2) n-SA' A' = H, CH_3 or S 

(corresponding to a number h) n being integer from 1 to 6

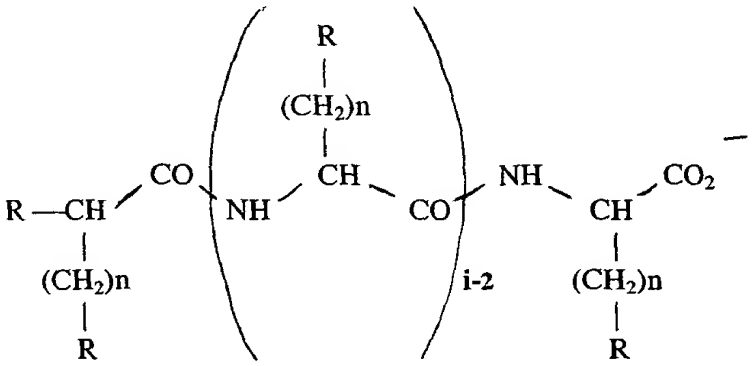
with
- $i = u + j + k + h$
- total number of α $NH_3^+ = p = u-q$
- total number of ω $NH_3^+ = j = f-(k + h)$
- total number of $NH_3^+ = m = p + j+1$

with the proviso that :

1) $u \geq i/2$

2) $m \geq i/2$

7. Oligomeric conjugate according to anyone of claims 1 to 6, wherein the oligomeric conjugate contains an oligomer of the following formula :

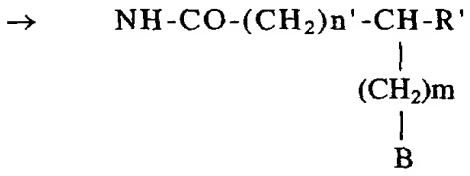


wherein

* i = degree of polymerization from 5 to 50, and particularly 10 to 40, and preferably 20,

* n = is an integer varying from 1 to 6, and preferably 4,

* R represents in a ratio of 50 % to 100 % (corresponding to u)



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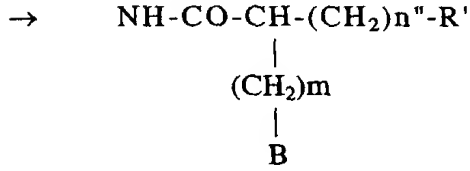

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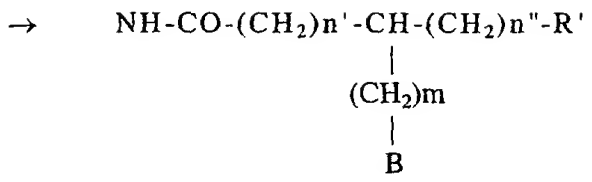


m is an integer varying from 1 to 6,

n' is an integer varying from 0 to 6,

n" is an integer varying from 0 to 6,

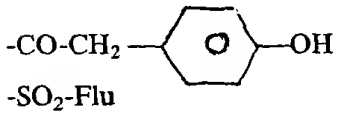
B is a weak base as defined according to anyone of claims 2 to 4,

R' represents NH_3^+ (corresponding to a number p),
or NH (corresponding to a number q) substituted by

$-CO-CH_3$

$-CO-(CHOH)rH$ r being an integer from 1 to 15, and preferably 1 to 7

$-CO-(CH_2)s-(CHOH)rH$ r being an integer from 1 to 15, and preferably 1 to 7, and s being an integer from 1 to 6, and preferably 6



$-SO_2$-Flu

-CO-Flu

-CS-NH-Flu

Flu being a fluorescent molecule

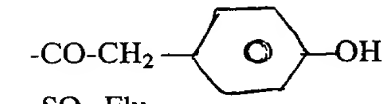
* R represents in a ratio of 0 % to 50 % (corresponding to $f : 0 < f \leq 1$)

- NH_3^+ (corresponding to a number j),
- NH (corresponding to a number k), substituted by

$-CO-CH_3$

$-CO-(CHOH)rH$ r being an integer from 1 to 15, and preferably 1 to 7

-CO-(CH_2)s-(CHOH)rH r being an integer from 1 to 15, and preferably 1 to 7, and s being an integer from 1 to 6, and preferably 6



-SO_2-Flu

-CO-Flu

-CS-NH-Flu

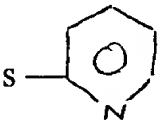
Flu being a fluorescent molecule

- H (corresponding to a number h)
- $(CH_2)_n$H, n being an integer from 1 to 6

(corresponding to a number h)

- $(CH_2)_n$-OH n being an integer from 1 to 6

(corresponding to a number h)

- $(CH_2)_n$-SA' A' = H, CH_3 or 

(corresponding to a number h) n being integer from 1 to 6

with
- i = u + j + k + h
- total number of α NH_3^+ = p = u-q
- total number of ω NH_3^+ = j = f-(k + h)
- total number of NH_3^+ = m = p + j + 1

with the proviso that :

1) $u \geq i/2$

2) $m \geq i/2$

8. Oligomeric conjugate according to anyone of claims 1 to 7, wherein the oligomeric conjugate contains an oligomer of the formula according to claim 7, wherein

i = 19 n = 4 (u) R= NH-CO-$(CH_2)n'$ -CH-$(CH_2)n''$-R'
|
$(CH_2)m$
|
B

wherein

n' = n" = 0

R' = NH^+_3

m = 1

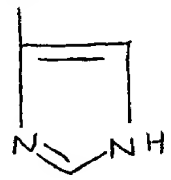
B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2

(f) R = NH^+_3

u = 12

j = 7



or

i = 19 n = 4 (u) R= NH-CO-$(CH_2)n'$ -CH-$(CH_2)n''$-R'
|
$(CH_2)m$
|
B

wherein

n' = n" = 0

R' = NH^+_3

m = 1

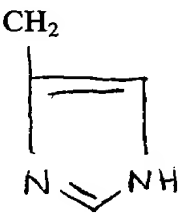
B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2

(f) R = NH^+_3

u = 16

j = 3



or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

n' = n" = 0

R' = NH^+_3

m = 1

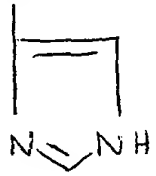
B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2

(f) R = NH^+_3

u = 19

j = 0



or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

n' = n" = 0

R' = NH^+_3

m = 1

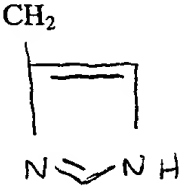
B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2

(f) R = $CO-CH_3$

u = 11

k = 8



or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

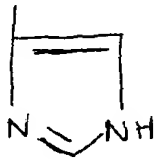
n' = n" = 0

R' = NH^+_3

m = 1

B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2



(f) R = $CO-CH_3$

u = 15

k = 4

or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

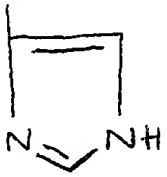
n' = n" = 0

R' = NH^+_3

m = 1

B = imidazole

R = NH-CO-CH- NH_3^+
|
CH_2



(f) R = CO-(CHOH)rH

r = 5

u = 12

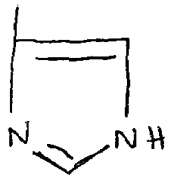
k = 3

or

i = 19 n = 4 (u) R= NH-CO-(CH_2)n' -CH-(CH_2)n"-R'
|
(CH_2)m
|
B

wherein

(q) n' = n" = 20
R' = NH-CO-CH_3
m = 1
B = imidazole
R = NH-CO-CH- NH-CO-CH_3
|
CH_2



(f) R = NH^+_3
u = 16
f = 4
k = 3

9. Composition containing at least one oligomeric conjugate according to anyone of claims 1 to 8, in association with at least one biological molecule, such as a peptide, an oligoside or an oligonucleotide or a mixture thereof.

10. Combined preparation containing as active substance the following individual components, in the form of a kit-of-parts :

- an oligomeric conjugate according to anyone of claims 1 to 8,
- at least one oligomeric conjugate according to anyone of claims 1 to 8, in association with at least one biological molecule, such as a peptide, an oligoside or an oligonucleotide, or a mixture thereof,

for the simultaneous, separate or sequential use, for the *in vitro*, the *in vivo* or the *ex vivo* transfer of said biological molecules into the cytosol and/or cell nucleus.

11. Use of an oligomeric conjugate according to anyone of claims 1 to 8, for the *in vitro*, the *ex vivo* or the *in vivo* intracellular transfer of biological molecules into the cytosol and/or in the cell nucleus.

12. Use of an oligomeric conjugate according to anyone of claims 1 to 8 or of a composition according to claim 9, or of a combined preparation according to claim10, for the intracellular the *in vitro*, the *ex vivo* or the *in vivo* transfer of a peptide, an oligoside or an oligonucleotide, or a mixture thereof, into the cytosol or/and in the cell nucleus.

13. Use of an oligomeric conjugate according to anyone of claims 1 to 8 or of a composition according to claim 9, or of a combined preparation according to claim10, wherein the cells are chosen among muscular, epithelial, endothelial, myeloid cells such as monocytes, macrophages and fibroblasts, leukocytes and granulocytes, osteoblasts as well as dendritic cells, stem cells, neuronal cells, or dermal cells.

14. Method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of an oligonucleotide, wherein an oligonucleotide and an oligomeric conjugate according to anyone of claims 1 to 8 or of a composition according to claim 9, or of a combined preparation according to claim10, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is :

- transfer of an antisense oligonucleotide in the cytosol and/or the cell nucleus where it binds and blocks the complementary mRNA sequence,
- or transfer of an oligonucleotide as activator into the cytosol where it depresses or activates a second messenger in the cytosol, or the corresponding gene in the nucleus,
- or transfer into the cytosol and/or the cell nucleus of oligonucleotides corresponding to a repetitive bacterial type DNA sequence with stimulating or immunodepressive activity,

- or transfer of an oligonucleotide in the cell nucleus where it binds to DNA and forms a triple helix leading to the inhibition of gene expression.

- or transfer into the cytosol and/or the cell nucleus of RNA oligonucleotide acting as decoys which inhibit gene expression by blocking the binding of regulatory factors to the authentic DNA region.

- or transfer into the cytosol and/or the cell nucleus of ribozymes (RNA oligonucleotides) which inhibit gene expression by cleaving the mRNA.

15. Method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of peptide, wherein a peptide and an oligomeric conjugate according to anyone of claims 1 to 8 or of a composition according to claim 9, or of a combined preparation according to claim 10, in particular wherein the peptide is an antigenic peptide, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is a transfer of said antigenic peptide in the cytosol of antigen presenting cells (macrophages, dendritic cells and B cells) where they are processed in proteosomes in order to bind to MHCI molecules, allowing the presentation of the antigenic epitope fixed on MHCI.

16. Method for the *in vivo*, the *in vitro* or the *ex vivo* transfer of an oligoside, wherein an oligoside and an oligomeric conjugate according to anyone of claims 1 to 8 or of a composition according to claim 9, or of a combined preparation according to claim 10, are(is) contacted with a medium containing cells to be transferred, under conditions such that there is a transfer of said oligoside into the cytosol and/or the cell nucleus.

17. Pharmaceutical composition, comprising as active substance at least an oligomeric conjugate according to anyone of claims 1 to 8, or of a composition according to claim 9, or of a combined preparation according to claim10, or in association with a pharmaceutically acceptable vehicle.

18. Use of an oligomeric conjugate according to anyone of claims 1 to 8, or of a composition according to claim 9, or of a combined preparation according to claim10, or for the preparation of a drug for use in the treatment of cancer, inflammatory or immunology diseases (such as graft rejection, allergy, auto-immunity) or infectious diseases.

19. Kit or case containing :

- an oligomeric conjugate according to anyone of claims 1 to 8, substituted by a protonable residue leading in a weak acid medium to a destabilization of cellular membranes, this oligomeric conjugate being able to comprise a recognition signal, which is previously fixed or not on the above-said conjugate, said recognition signal being dependent upon the cell to target,
- at least one biological molecule to transfer,
- optionally reagents enabling the possible binding of the recognition signal on the above-said oligomeric conjugate,
- optionally reagents enabling the formation of a composition according to claim 9, or of a combined preparation according to claim 10,
- reagents enabling the transfer of the biological molecule in the cytosol and/or the cell nucleus.

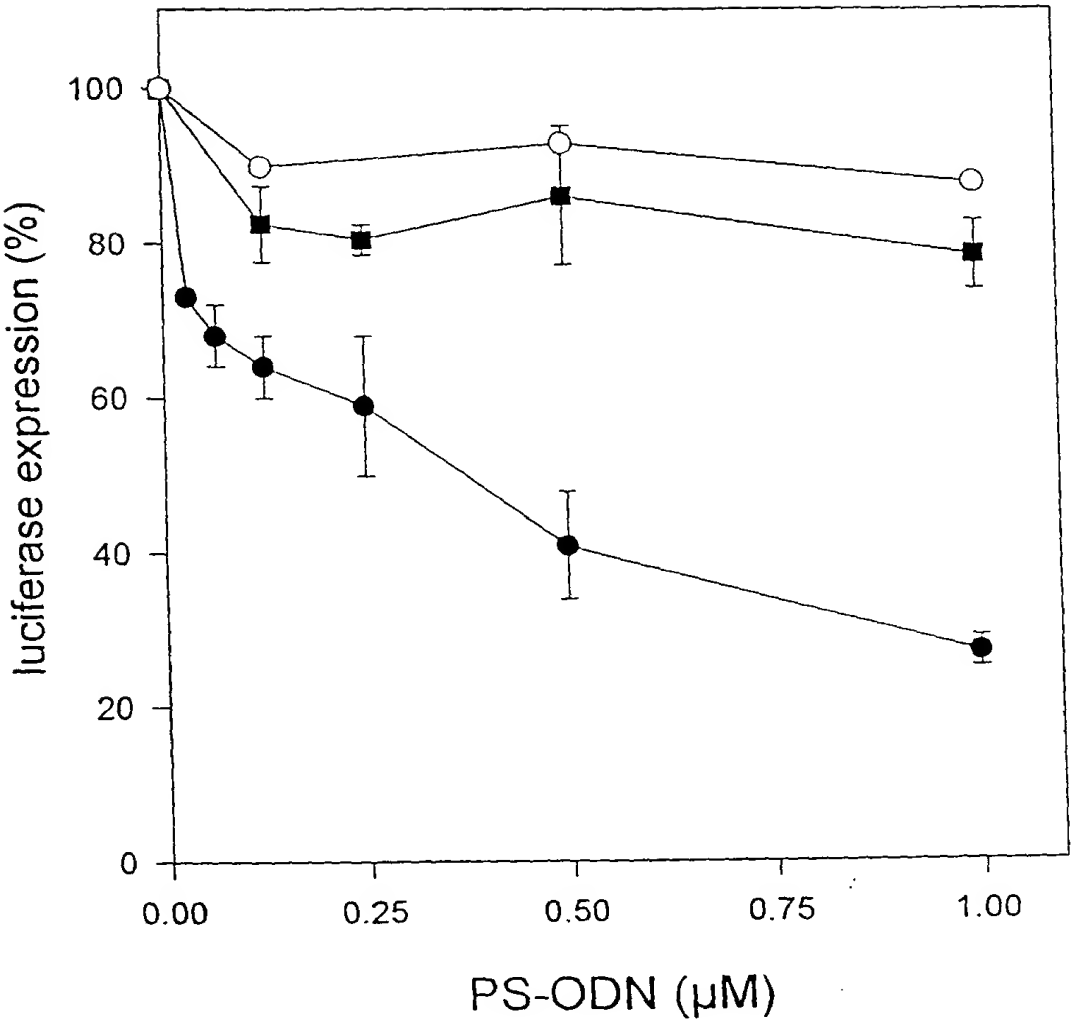

100
80
60
40
20
0
luciferase expression (%)
0.00
0.25
0.50
0.75
1.00
PS-ODN (µM)

Figure 1

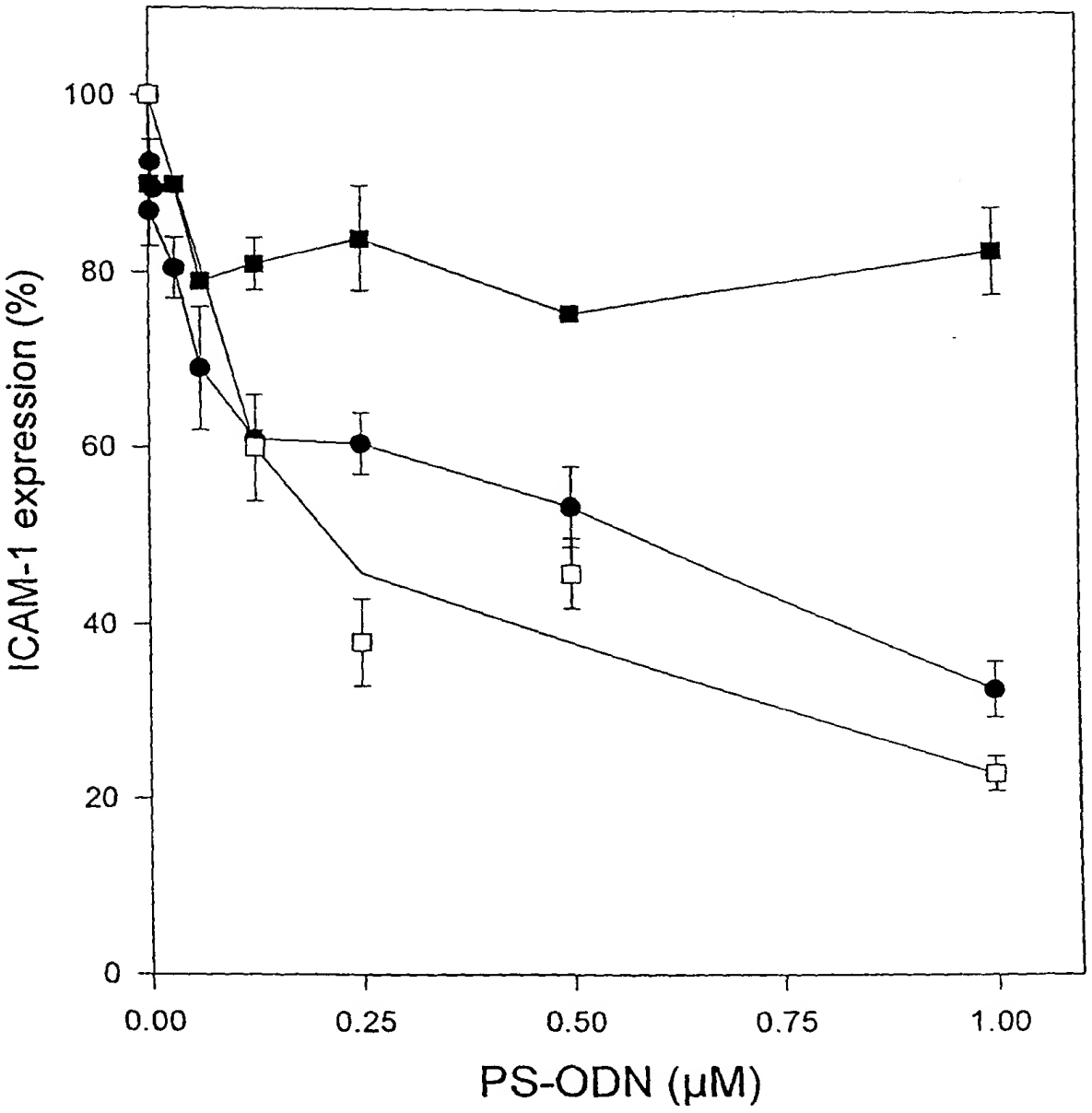


Figure 2

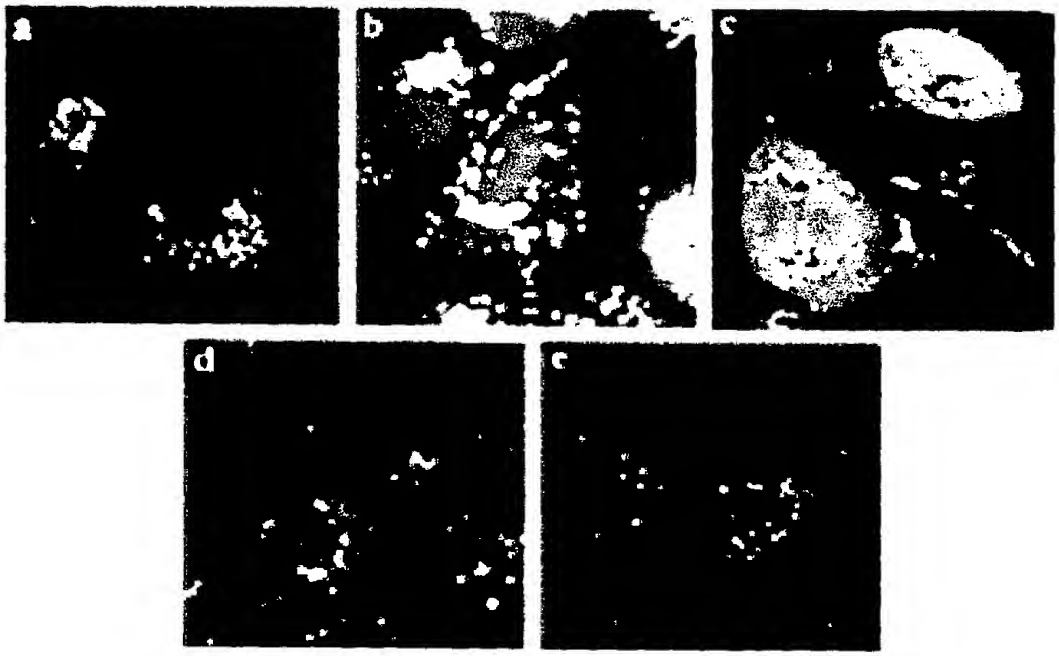


Figure 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 99/08980

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/11 A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 22610 A (IDM IMMUNO DESIGNED MOLECULES ;MIDOUX PATRICK (FR); MONSIGNY MICHE) 28 May 1998 (1998-05-28) cited in the application * see in particular the claims; and Figures 1,5 *	1-7,9-19
A	WO 98 19710 A (SCHACHT ETIENNE HONORE ;ULBRICH KAREL (CZ); SEYMOUR LEONARD CHARLE) 14 May 1998 (1998-05-14) *see p.39,l.30 - p.40; claims 1,6-8*	1-19
A	US 5 627 270 A (HATZENBUHLER NICOLE T ET AL) 6 May 1997 (1997-05-06) * see claims 1,3; col.71,l.36-38 *	1-19

-/--

[X] *Further documents are listed in the continuation of box C.*

[X] *Patent family members are listed in annex.*

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2000	17.03.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Isert, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 727 223 A (HISAMITSU PHARMACEUTICAL CO) 21 August 1996 (1996-08-21) * see the claims; Figures 8-10; page 7 *	1-19
A	GOTO T. ET AL: "A novel approach for gene medicine;synthetic poly-L- lysine /serine copolymer enhances bioactivity of antisense oligonucleotides." NUCLEOSIDES AND NUCLEOTIDES, (1997) 16/7-9 (1609-1615). REFS: 10 ISSN: 0732-8311 CODEN: NUNUD5, XP002108844 United States *see in particular abstract and introduction *	1-19
A	MIDOUX P ET AL: "Membrane permeabilization and efficient gene transfer by a peptide containing several histidines." BIOCONJUGATE CHEMISTRY, (1998 MAR-APR) 9 (2) 260-7. JOURNAL CODE: A1T. ISSN: 1043-1802., XP002108845 United States *see in particular the abstract*	1-19

INTERNATIONAL SEARCH REPORT

Inte. ..ational application No.
PCT/EP 99/08980

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 11-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

The conjugate according to claims 1-8 is not clear in view of the broad and vague definition of both the "monomeric component" and the "protonable residues". The search had to be restricted for economic reasons and was limited to the worked examples and to the general idea underlying the application.
See Articles 5 and 6 PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNA[redacted]L SEARCH REPORT

Information on patent family members

Inte[redacted]nal Application No
PCT/EP 99/08980

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9822610	A	28-05-1998	FR	2755976	A	22-05-1998
			AU	5123998	A	10-06-1998
			EP	0946744	A	06-10-1999
WO 9819710	A	14-05-1998	AU	4873997	A	29-05-1998
			EP	0941123	A	15-09-1999
US 5627270	A	06-05-1997	US	5693769	A	02-12-1997
			US	5571795	A	05-11-1996
			US	5338837	A	16-08-1994
			AU	687557	B	26-02-1998
			AU	2358295	A	16-11-1995
			CA	2188320	A	02-11-1995
			EP	0756601	A	05-02-1997
			JP	9512270	T	09-12-1997
			NZ	284902	A	22-09-1997
			WO	9529186	A	02-11-1995
			US	5795870	A	18-08-1998
			US	5780444	A	14-07-1997
			AT	166577	T	15-06-1998
			AU	3278593	A	19-07-1993
			BR	9206927	A	21-11-1995
			CA	2117332	A	24-06-1993
			DE	69225719	D	02-07-1998
			DE	69225719	T	24-12-1998
			EP	0618800	A	12-10-1994
			ES	2118932	T	01-10-1998
			HU	70743	A	30-10-1995
			IL	104089	A	09-05-1999
			JP	7503708	T	20-04-1995
			NO	942165	A	01-08-1994
			NZ	246448	A	28-07-1998
			PL	171131	B	28-03-1997
			WO	9311772	A	24-06-1993
			US	5455335	A	03-10-1996
EP 0727223	A	21-08-1996	AU	681192	B	21-08-1997
			AU	7706994	A	18-04-1995
			US	5912300	A	15-06-1999
			CA	2172974	A	06-04-1995
			WO	9509009	A	06-04-1995